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ON THE LIFE HISTORY OF *SYNTHESIOMYIA NUDISETA* VAN DER WULP (DIPTERA, MUSCIDAE), A MYIASIS-PRODUCING FLY

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(With 19 Figures in the Text)

I. INTRODUCTION

Townsend (1935) has described the egg of *Synthesiomyia nudiseta* and recorded the interesting fact that the larva forms a cocoon. The larval stages and the puparium do not appear to have been described. That this species was not among the larvae of some unknown muscids described by Banks (1912) is evident from the figures of the posterior spiracles. The present communication provides an account of all the early stages.

II. SYSTEMATICS*

Synthesiomyia (*Cyrtoneura*) *nudiseta* van der Wulp (1883) is the valid name for the genotype *S. brasiliana* Brauer & Bergenstamm, 1893 (in Aldrich, 1905). The synonymy was discussed by Bezzi (1911) who published also a list of synonyms (1923).

Séguy (1937) believes that *Cyrtoneura analis* Macquart should be included in the genus *Synthesiomyia* although the type specimen is defective; but Bezzi (1922) has pointed out that *Cyrtoneura analis* differs in the hairy eyes and black first antennal joint; in *Synthesiomyia* the eyes are bare and the first antennal joint is orange. *S. nudiseta* is, in fact, the only known species of the genus. Modern definitions of the fly are provided by Curran (1934), Townsend (1935), Séguy (1937) and Van Emden (1939).

III. BIOLOGY

S. nudiseta has a wide distribution in the warmer parts of the globe. Bezzi's (1923) list is incomplete and probably out of date, since India is not mentioned. In Calcutta it is an infrequent domestic fly. A few specimens were also captured in the laboratory, possibly attracted by putrid meat. Among these were gravid females which provided the materials described in this paper. *S. nudiseta* breeds in a variety of decomposing animal and vegetable matter: such as human cadavers (Townsend, 1935) and decayed cotton seed; locusts (Engel, 1931, in Séguy, 1937); corpses of rats (Aders, 1917); and carrion and faeces (Aubertin & Buxton, 1934). In Calcutta, during an extended survey of the breeding habits of flies, the authors bred it once only, from kitchen refuse consisting of the leavings from prawns. The larvae also cause secondary human myiasis

* Specimens of the fly dealt with in the present communication were sent to Dr C. H. T. Townsend who suggested the possibility of a new species, but owing to paucity of material and possible desiccation could not offer a definite opinion. Unfortunately, the present war made it difficult to send further specimens. But as the fly agrees with the descriptions of *Synthesiomyia nudiseta* provided by Dr F. Van Emden, who also informed us that this species has been taken before in Calcutta, it is considered that this account pertains to the same species.

(Coia, in Aubertin & Buxton, 1934). Lutz (in Séguy, 1937) states that the fly can disseminate the young larvae of *Dermatobia hominis*.

IV. Life history

The life history was followed in the laboratory by rearing adults from eggs deposited by wild-caught gravid females on slightly putrid meat. Sand was provided for pupation.

A female caught (19 August 1938) in the laboratory deposited 255 eggs the following morning (20 August 1938). The eggs had not hatched by 4 p.m. that day, but the next morning (21 August 1938) second-stage larvae were observed, 24 hr. after deposition. Third-stage larvae were observed 48 hr. after deposition (22 August 1938). This stage, including the prepupa, occupied 6 days, when pupation took place (28 August 1938). Adults of both sexes emerged 7 days later (4 September 1938). Thus the development from egg to adult in the summer season in Calcutta occupies 15 days. A second batch of eggs from another female was laid 5 days after the first, indicating that maturation of eggs takes 5 days and that the whole cycle from egg to egg is completed in 20 days in summer. First-stage larvae were recovered from another batch of eggs 15 hr. after deposition. The meat did not present that typical maggot-eaten appearance since the larvae fed at the surface, though under the meat, and did not burrow like *Chrysomyia* spp. and *Sarcophaga* spp. This is possibly related to the absence of the recess in which the posterior spiracles in calliphorids are lodged.

Cocoons which are present in this species are formed as was already described by Keilin (1919) in another muscid. They consist, briefly, of the secretion, most likely from the salivary glands, of a frothy fluid which soon solidifies as a whitish pellicle, in which sand grains are cemented.

V. Early stages

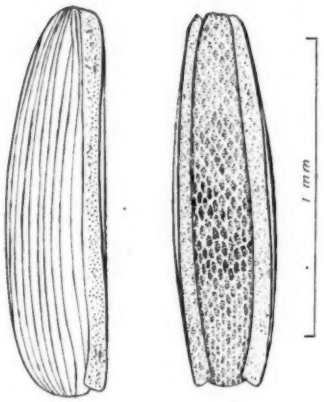
Egg (Figs. 1, 2)

The oval egg is flat dorsally, convex ventrally, and narrowed towards the micropylar end. The chorion is expanded into broad, dorso-lateral flanges, and is marked dorsally between the flanges with a reticulate pattern, but the rest of its surface shows longitudinal striae. The egg measures about 1·3 mm. in length.

The egg when deposited lies on its convex surface. The larva escapes by a small slit which appears either on the right or left side near the anterior end, and just below the flange. Generally in muscids the chorion splits along the mid-dorsal line.

First-stage larva (Figs. 3–5)

The larva on hatching is about 1·5 mm. long and grows to 3 mm. before the first moult. A feature of the larva is the presence of tubercles in the anal region of the eighth abdominal segment (Fig. 3): one (*tub.p.*) is median, and posterior to the anus, and bears dorsally directed spines; three (*tub.* 1, 2 and 3) are paired, ventro-lateral in position, and are without spines. There is also a spined area above the median tubercle. Spines are present at the anterior, ventral margin of the first thoracic segment (Fig. 5, *th.* 1) and on the transverse ventral ridges of the abdominal segments, those of the first seven segments being directed posteriorly, and those of the eighth, anteriorly (Fig. 3, *v.r.*). The spines of the ventral ridges are arranged in transverse rows, those of the anterior row



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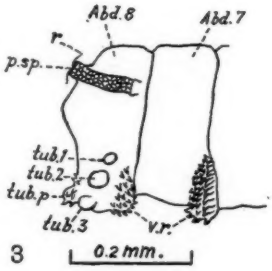
Figs. 1, 2. Egg, left lateral and dorsal views respectively.



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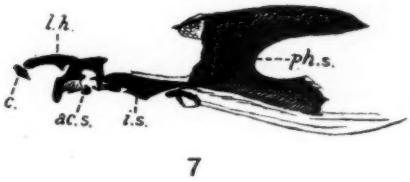

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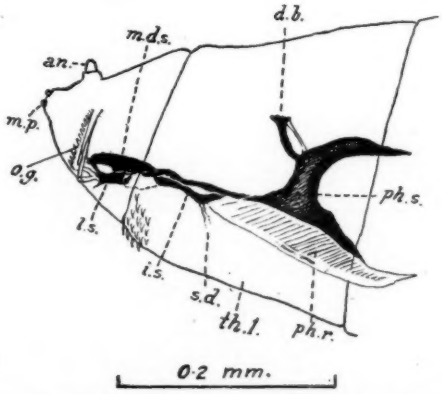

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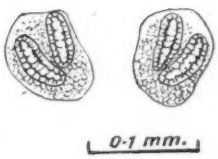

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Figs. 3–5. First-stage larva, posterior end (lateral view), posterior spiracles (rays not shown) and anterior end (lateral view) respectively.

Figs. 6, 7. Second-stage larva, posterior spiracles and cephalopharyngeal skeleton (drawn to same scale as Fig. 3) respectively.

with large and somewhat elongate bases, and those of the rows behind, diminishing in size backwards, with longer tips relative to the bases.

The larva is metapneustic. The spiracles (Figs. 3, *p.sp.* and 4) project a little and are heart-shaped. There are very delicate rays (*r.*) round the slits. On the head (Fig. 5), the single-jointed antennae (*an.*), maxillary palps (*m.p.*), and oral grooves (*o.g.*), two in number on each side, are distinct. The anterior margin of the first groove is fringed. The cephalopharyngeal skeleton consists of the usual sclerites: a pharyngeal sclerite (*ph.s.*) with well-developed dorsal bridge (*d.b.*), oblique pharyngeal ridges (*ph.r.*), and anterior prolongations* meeting the blade-like, median, dorsal sclerite (*m.d.s.*); a ventral H-shaped intermediate piece (*i.s.*); and paired, lateral sclerites (*l.s.*) with fine anterior processes. The exact relationship between these parts is difficult to determine.

Second-stage larva (Figs. 6, 7)

The larva of the second stage grows from 3 to 7 mm. The posterior tubercles are better developed in this stage, and all are spined. Those of the lateral tubercles are colourless and more fleshy. There is a transverse, spined area above the median tubercle. Complete belts of spines are observed at the anterior margins of the three thoracic segments and on the first two abdominal segments. The ventral ridges are similar to those of the first-stage larva except that the spines of the anterior row are larger.

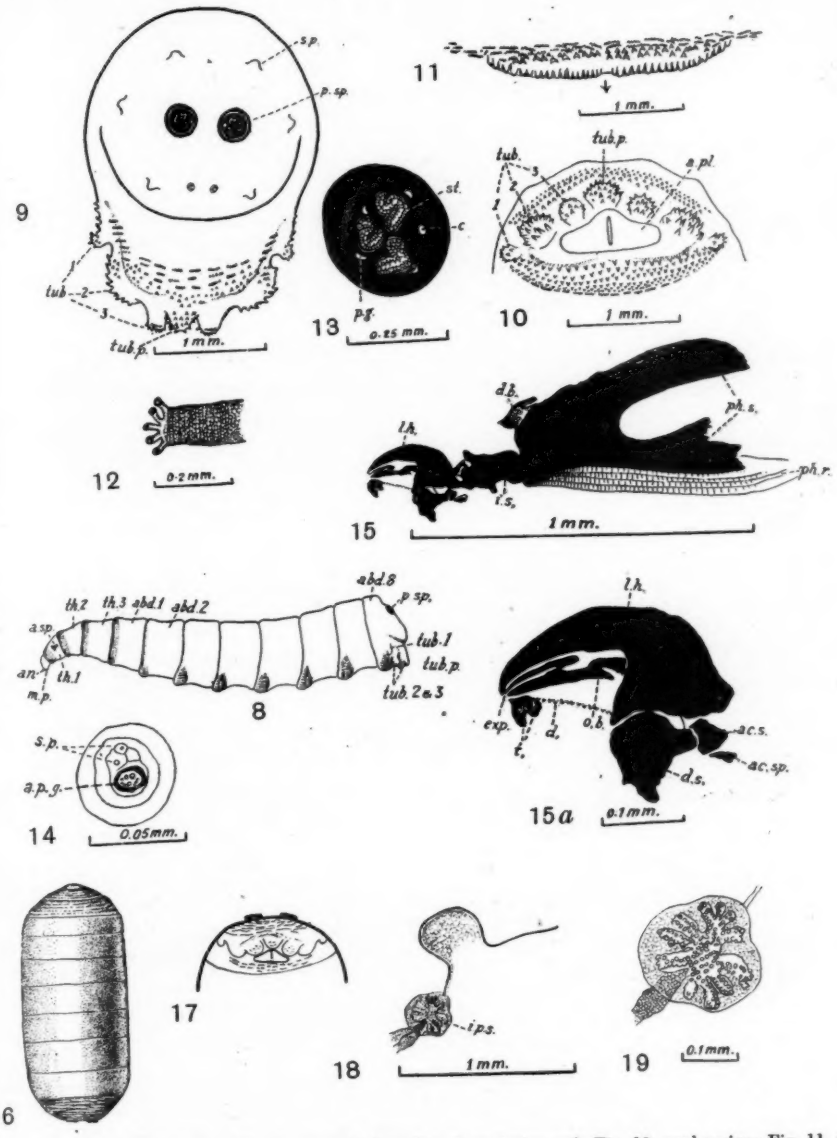
The larva is now amphipneustic. The anterior spiracles have 5–7 processes. The posterior spiracles (Fig. 6) possess two wide, convergent slits with relatively thick peritremes. Cuticular strands from the peritreme cross the slits.

The oral grooves are more numerous, and antennae and palps well formed. The disposition of the sensory papillae of the palps is best seen in the third-stage larva. The cephalopharyngeal skeleton (Fig. 7) now consists of three main parts: a pharyngeal sclerite (*ph.s.*), a robust and heavily chitinized intermediate piece (*i.s.*), and an anterior assemblage of sclerites. This last consists of paired lateral hooks (*l.h.*) with broad ventral expansions, a pair of small accessory, vertical sclerites (*ac.s.*) behind these expansions, and an anterior pair of lanceolate teeth.

Third-stage larva (Figs. 8–15*a*)

The young third-stage larva is about 7 mm. long, and when mature it grows to 15 mm. It is creamy white and relatively thick (Fig. 8). The posterior end (Fig. 9) presents a prominent disk bearing the black, posterior spiracles (*p.sp.*). Six large, and two small sensory papillae (*s.p.*) are situated around the margin of the disk. The definitive appearance of the spinulose tubercles (Figs. 8–10, *tub.*) is striking, the description given above of these parts in the second larva fitting equally well here. The anal plate (Fig. 10, *a.pl.*) is triangular. There is a belt of spines at the anterior margins of the three thoracic (Fig. 8, *th.* 1–3), and the first and second abdominal segments (*abd.* 1, 2), though only poorly developed on the last. On abdominal segments 2–7, the large spines of the anterior row of the ventral ridges (Fig. 11) are fleshy with elongate bases. Behind this row, the spines are not arranged in such an orderly manner and have fine, dark points. In all these regions the smaller spines are commonly arranged in linear groups.

* An apparent oblique joint in the course of the prolongations suggests that these are not entirely derived from the pharyngeal sclerite, but partly from posterior extensions of the median, dorsal sclerite.



Figs. 8–15*a*. Third-stage larva. Fig. 8, general view; Fig. 9, posterior end; Fig. 10, anal region; Fig. 11, ventral ridge (arrow directed towards anterior end); Fig. 12, anterior spiracle; Fig. 13, left posterior spiracle; Fig. 14, right maxillary palp (apical view); Fig. 15, cephalopharyngeal skeleton; Fig. 15*a*, left lateral hook and associated sclerites.

Figs. 16–19. Puparium and pupa. Fig. 16, puparium; Fig. 17, anal region of pupa (not drawn to scale); Fig. 18, pupal spiracle; Fig. 19, intra-pupal spiracle.

The anterior spiracles (Figs. 8, *a.sp.* and 12) have 4–7 processes. The posterior spiracles (Fig. 13) are rounded and black owing to dense chitinization; they are separated by about three-fourths the width of a plate. The cicatrices (*c.*) are evident, but the openings of the peristigmatic glands (*p.g.*) are not always clearly distinguished owing to the chitinization of the plate. The stigmata (*st.*) are irregularly S-shaped, and somewhat radially arranged.

The antennae are two-jointed. The maxillary palps (Fig. 14) are each surmounted by a low, oval, disk-like joint bearing the sensory papillae (*ap.g.*). There are two more papillae (*s.p.*) situated apart from the main apical group, on the general surface of the palp, within areas bounded by cuticular lines. The oral grooves are prominent and numerous, and there is no fringe as in the first-stage larva. Labial palps may now be detected ventrally behind the oral funnel.

The cephalopharyngeal skeleton (Figs. 15, 15*a*) is typically muscid. The pharyngeal sclerite (*ph.s.*) is provided with longitudinal ridges (*ph.r.*). The intermediate piece (*i.s.*) is perfectly H-shaped in dorsal or ventral view, exceedingly robust, and ankylosed to the pharyngeal sclerite. The lateral hooks (*l.h.*) are equal in length and closely apposed. Arising from the inner surface of the base of each hook is an accessory sclerite, the oral bar (*o.b.*). Between this sclerite and the hook is a chitinous strip (*exp.*) which appears to be an expansion of the oral bar. The dentate sclerites (*d.s.*) form a median ventral arch. Anteriorly there is a pair of large teeth (*t.*) each with an outer, and an inner cusp, which are often slightly bifid. Running from the base of the teeth to the basal region of the hooks, on both sides, and set internally on the wall of the oral cavity, is a row of denticles (*d.*). Behind and internal to the dentate sclerites, there is a pair of smaller vertical plates (*ac.s.*). Posterior to these again, and best seen in very late stages, is a pair of chitinous spicules (*ac.sp.*) of doubtful relationship.

Puparium (Figs. 16–19)

The puparium (Fig. 16), which is enclosed in a cocoon, is brown, somewhat transparent, and is 7–8 × 3 mm. The spined larval tubercles and anal plate may be discerned, though now shrivelled (Fig. 17). Pupal spiracles do not project externally as horns but are represented internally in the anterior thoracic region of the pupa by round, chitinous tubercles (Fig. 18). Intra-pupal spiracles (Figs. 18. *i.p.s.* and 19) are present: their papillae are arranged in a foliate pattern.

Emergency of the fly takes place by the usual frontal and transverse splits, the latter passing through the first abdominal segment.

VI. Conclusion and summary

Observations on the life history of *Synthesiomyia nudiseta* van der Wulp are recorded. The larval stages occupy 8 days and the pupal 7 days in summer in Calcutta. The early stages of this fly, all of which are described, are essentially muscid in type. The distinctive features of the third-stage maggot are the rounded, densely chitinized, posterior spiracles with irregular S-shaped, radially placed slits; the row of denticles set between the anterior teeth and the base of the lateral hooks; and the chitinous spicules posterior to the dentate sclerites. The bicuspid anterior teeth are possibly also very distinctive. Less characteristic are the spinulose anal papillae, the triangular anal plate and the

additional sclerites in the cephalopharyngeal skeleton. The nature of the latter confirms what is known of the breeding habits of the fly. The semi-carnivorous habits of the larva are indicated by the combined presence of pharyngeal ridges, accessory sclerites, and median ventral arch (Keilin, 1912, 1915, 1917; Keilin & Tate, 1930; Muirhead Thomson, 1937). The puparium is encased in a cocoon.

We have to thank Dr C. H. T. Townsend, Sao Paulo, Brasil, for stimulating interest in this fly; Dr F. van Emden, Imperial Institute of Entomology, London, for descriptions of the adult; and Dr Baini Prashad, Director, Zoological Survey of India, for many practical facilities of the Library and Entomology Department of the Indian Museum, Calcutta, the staffs of which have always shown us the utmost kindness.

Key to lettering

a.pl. anal plate; *a.sp.* anterior spiracle; *abd.* abdominal segments 1–8; *ac.s.* accessory sclerite; *ac.sp.* accessory spicule; *an.* antenna; *ap.g.* apical group of sensory papillae; *c.* cicatrix; *d.* denticle; *d.b.* dorsal bridge; *d.s.* dentate sclerite; *exp.* expansion of oral bar; *i.p.s.* intra-pupal spiracle; *i.s.* intermediate sclerite; *l.h.* lateral hook; *l.s.* lateral sclerite; *m.d.s.* median dorsal sclerite; *m.p.* maxillary palp; *o.b.* oral bar; *o.g.* oral groove; *p.g.* opening of peristigmatic gland; *p.sp.* posterior spiracle; *ph.r.* pharyngeal ridge; *ph.s.* pharyngeal sclerite; *r.* ray; *s.d.* salivary duct; *s.p.* sensory papilla; *sp.* spiracles; *st.* stigma; *t.* tooth; *th.* thoracic segments 1–3; *tub.p.* 1, 2 and 3, posterior, and three lateral tubercles; *v.r.* ventral ridge.

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ON THE CULTURING OF *RHABDITIS TERRESTRIS* N.SP.

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(With 6 Figures in the Text)

I. Introduction

A general investigation of the effect of various environmental factors upon a free-living soil nematode was undertaken, using as experimental material a species of *Rhabditis* which had been obtained from rotting earthworms. Cultures were started from a single mature female, and when identification was attempted, it was evident that the species did not agree with the description of any known form. Hence the species is here described as new.

II. *Rhabditis terrestris* n.sp., specific description

Most specific descriptions are based upon a consideration of animals collected in the field. In this case, the description is based upon animals raised in cultures, and it is difficult, from a consideration of these individuals, to estimate the range of variability to be expected in the field. Thus certain of the culture conditions were abnormal in the sense that continuous culturing was not possible. On the other hand, in those cultures in which prolonged and successful culturing has been demonstrated (standard cultures, see p. 249) no males were ever observed. It has therefore been thought desirable to give the observed range of the dimensions and of the proportions of the parts under all culture conditions, irrespective of whether continuous culturing was possible or not, and also the range under the standard culture conditions which are described on p. 249 (see Table 1).

Features of diagnostic importance

Both sexes (see Fig. 1)

Cuticle: Thin, with extremely fine striations about 4μ apart. These striations are visible only with difficulty in the male. *Lips:* Three, fairly distinct, each with a single papilla. *Buccal tube:* Of the generic type, about 20μ long, with tri-radiate lumen, without teeth or ridges, with the usual constriction and terminal expansion. *Oesophagus:* With a well-marked anterior bulb. *Nerve cord:* Approximately midway between the two oesophageal bulbs. *Lateral organs:* Not observed except possibly under certain experimental conditions.

Males

Occurrence: Usually absent from the cultures and never forming more than 2% of the population. *Arrangement of reproductive system:* Single, anterior, reflexed. *Bursa:* Of the Peloderian type, with the tail filament projecting about 10μ beyond its edge. Typically there are nine pairs of bursal rays, of which three pairs are pre-anal, three pairs just post-anal, and the remaining three pairs at the posterior end of the bursa. The typical bursal formula is: (1.–3.), (4.–6.),+(7.–9.). The middle rays (5) are the only ones extending right to the edge of the bursa. Sometimes the two most posterior rays (1 and 2) are very close together, and only distinguishable from each other with

difficulty. In one individual the left side of the bursa had lost the second and ninth rays, while the right side remained typical (see Figs. 2–5). *Spicules:* Curved ventro-medially, the left slightly longer than the right. With longitudinal ridges on their surfaces, supported by small accessory pieces.

Females (Fig. 4)

Arrangement of reproductive system: Reflexed, opposed, with the vulva near the middle of the body. *Rectal glands:* Large, at the junction of the intestine and rectum. *Phasmids* (of the American workers)*:* Just posterior to the anus, connected by fine ducts to two papillae about 55 μ posterior to the anus.

Table 1. *The range of values for the dimensions and proportions of the parts*

	Males	Females	
	Range under all culture conditions	Range under all culture conditions	Range under standard culture conditions (see p. 249)
Total length	1330–626	2580–930	1400–980
Length buccal cavity	30– 24	34– 28	30– 28
Length oesophagus	208–192	256–196	218–214
Length tail	56– 36	184–116	150–128
Anterior to nerve ring	140–132	200–146	162–148
Anterior to vulva	—	1336–510	720–518
Width at nerve ring	56– 36	80– 46	60– 46
Width at base oesophagus	60– 38	86– 48	64– 48
Width at mid-body	70– 42	114– 52	80– 56
Width at vulva, including lips	—	136– 54	82– 58
Width at anus	38– 22	48– 22	34– 22
α	19·0–14·9	19·1–15·5	17·1–16·9
β	6·4– 3·3	10·1– 4·2	6·4– 4·6
γ	23·8–17·4	15·7– 7·7	9·3– 7·7
V	—	58–49%	53–51%

Dimensions in the above table are all given in μ to the nearest 2 μ. The proportions of the parts are given by de Man's symbols (1910), where α is the length of the body divided by its greatest breadth, β is the total length divided by the length of the oesophagus, and γ is the total length divided by the length of the tail. The symbol V expresses the distance of the vulva from the anterior end as a percentage of the total length, as first used by Micoletzky (1921).

III. CULTURE METHODS

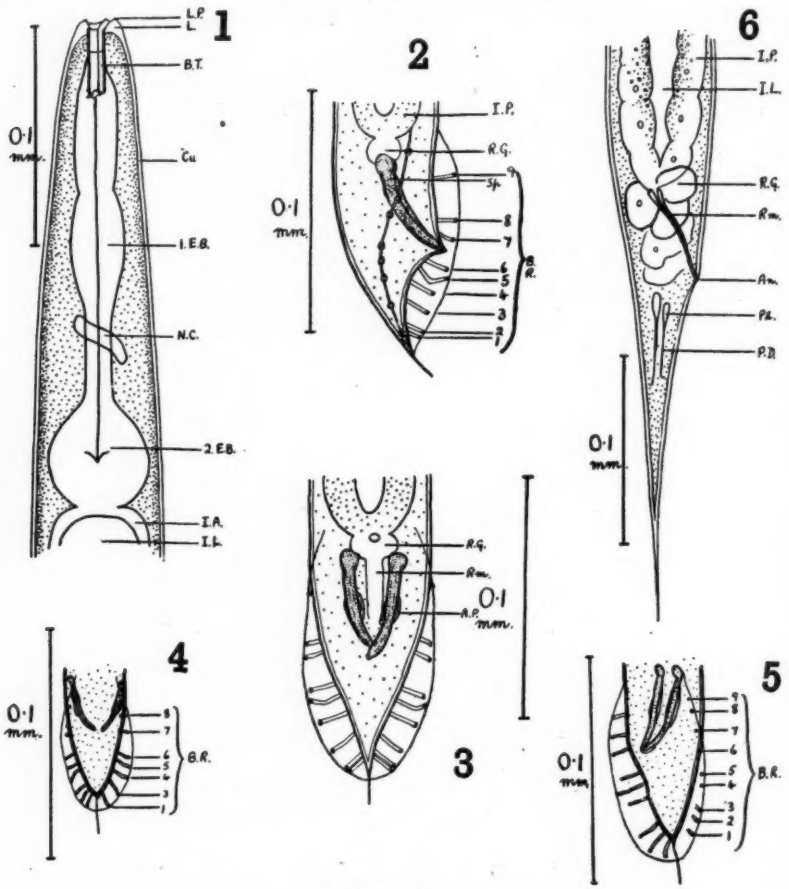
During the development of the standard culture method (see p. 249), the effects of variations in the amount of food supplied to the nematodes, in the salinity, and in the viscosity of the culture media, were investigated.

(*a*) *Food*

Approximately 100 c.c. of earthworms were boiled in 250 c.c. of tap water for 3 hr., and the mixture diluted to 300 c.c. This was allowed to settle for 24 hr. and the supernatant liquid drawn off, sterilized in an autoclave, and stored under sterile conditions. Various dilutions of this earthworm broth were used as culture media in several series of preliminary culture experiments. In each of these mature females were used to begin the cultures.

The results obtained show that there were two different types of optimal conditions, depending on the criteria of success. Mature individuals were produced most rapidly in

broth concentrations of between 20 and 60%, since below 20% many larvae died before attaining maturity, while above 60% egg laying was postponed and the individuals grew markedly larger. Thus the largest individuals were produced in concentrations above 80%. Since large numbers of individuals were required, a broth concentration of 33% was found to be most suitable and was employed in the standard medium (see p. 249).



Figs. 1–6.

Fig. 1. Lateral view of the anterior end of a female. Fig. 2. Ventro-lateral view of bursa. Fig. 3. Ventral view of bursa. Fig. 4. Ventral view of asymmetrical bursa. Fig. 5. Ventro-lateral view of bursa. Fig. 6. Lateral view of tail of female. *An.* anus; *A.P.* accessory piece; *B.R.* bursal rays, numbered from posterior, 1–9; *B.T.* buccal tube; *Cu.* cuticle; 1*E.B.* first oesophageal bulb; 2*E.B.* second oesophageal bulb; *I.A.* anterior part of intestine; *I.L.* lumen of intestine; *I.P.* posterior part of intestine; *L.* circumoral lips; *L.P.* papillae on lips; *N.C.* nerve cord; *P.D.* ducts of phasmids; *Ph.* phasmids; *R.G.* rectal glands; *Rm.* rectum; *Sp.* spicules.

(*b*) *Salinity*

Three series of experiments were performed to examine the effects of salinity upon culturing, and in these the salinities of the media were increased by: (i) the addition of NaCl to the standard medium; (ii) using different concentrations of sea-water containing

worm broth; (iii) using different concentrations of a mammalian Ringer solution containing de Witte's peptone.

In each case mature females from standard cultures were used to start the experiments, and in each case precautions were taken to maintain constant salinities and give equivalent amounts of food to the cultures. In each series of experiments, the rate of reproduction was most rapid in the medium whose salinity was equivalent to 0·03 *M* NaCl, and decreased with increasing salinity. Finally, a limiting concentration was reached (salinity equivalent to about 0·34 *M* NaCl), at which cultures could only be continued with difficulty and above which the nematodes died out. The above experiments were repeated with animals cultured for three generations at high salinity (equivalent to 0·30 *M* NaCl). The close similarity in the results obtained showed that the 'strains' of animals produced in media of high salinity show no permanent adaptation to these conditions.

(*c*) *Viscosity*

In the worm broth cultures described previously, the nematodes moved almost continuously by means of undulations of the body, but these undulations produced but little directive movement of the body as a whole. On increasing the viscosity of the medium by the employment of agar solutions (0·75–0·35 %) rapid directive movement resulted.

(*d*) *The standard method of culturing*

The standard culture medium finally employed had the following composition: 0·6 % agar solution, 2 parts; and earthworm broth, 1 part.

The standard method of culturing was to introduce 1·5 c.c. of a culture containing large numbers of 2-day-old larvae into 15 c.c. of the above medium in a 10 cm. Petri dish. The larvae grew gradually to become mature in from 4 to 11 days depending on the laboratory temperature. A large number of eggs were then laid, and when the larvae produced from them had become very numerous, these were used to begin a new culture. In this way over 100 successive generations have been raised successfully.

IV. VARIATIONS PRODUCED IN THE SPECIES BY ALTERATIONS IN THE CULTURE METHODS

(*a*) *Variations in the amount of food*

When a considerably larger number of larvae than that employed in the standard culture method was used to begin a culture (e.g. 50 % greater), or if the amount of worm broth was proportionately reduced, similar effects were observed. Both types of cultures can be described as 'starved' or 'overcrowded'. Similarly, when a much smaller number of larvae was used (e.g. 50 % of the standard number), or if the amount of worm broth was proportionately increased, the cultures can be described as 'well fed'. A special case of this occurs when mature females from 'well-fed' cultures are transferred singly to the amounts of standard culture medium employed in the standard method. These cases are discussed below under the heading of 'Cultures from isolated individuals'.

The mean dimensions of groups of animals from standard cultures and from the various types of cultures described above, are given in Table 2, cols. 1–4. From these results it can be concluded that increasing the amount of food available for the worms produces the following effects: (1) An increase in the total length of the body. (2) A propor-

tionately greater increase in the distance between the second oesophageal bulb and the anus, i.e. in the length of the intestine. (3) Proportionately smaller increases in the lengths of the remaining portions of the body (i.e. buccal cavity, oesophagus, and tail). (4) A general increase in the thickness of the body. This is proportionately less than the increase in the length of the body as indicated by the increase in the values of α.

Other more general effects produced by increasing the amount of food were as follows: (1) A marked increase in the opacity of the intestinal cells owing to increases in the number of vacuoles and granules present. (2) A decrease in the width of the intestinal lumen. (3) An increased growth rate of the larvae. (4) A marked increase in the longevity of the adults. (5) An increase in the time required to complete a generation. (6) An increase in the number of eggs laid by an individual female. (7) An increased

Table 2. *Mean dimensions and proportions of the parts of groups of females raised in different types of cultures*

	Worm broth agar cultures				Cultured in concentrated balanced salt solution with de Witte's peptone
	Cultures from isolated individuals	Well-fed cultures	Standard cultures	Starved cultures	
	Col. 1	Col. 2	Col. 3	Col. 4	Col. 5
Total length	2150	1388	1200	1056	1690
Length buccal cavity	32	30	30	30	32
Length oesophagus	232	206	210	214	208
Length tail	164	148	130	126	120
Anterior to nerve ring	190	160	154	150	172
Anterior to vulva	1110	700	640	556	894
Width at nerve ring	70	58	54	48	70
Width at base oesophagus	78	62	56	50	80
Width at mid-body	108	80	68	60	96
Width at vulva, including lips	118	84	72	62	110
Width at anus	42	32	26	24	44
α	18·1	16·5	16·7	17·0	15·5
β	9·3	6·7	5·7	4·9	8·2
γ	13·1	9·4	9·2	8·4	14·1
V	52%	50%	53%	53%	53%

All dimensions in the above table are given in μ to the nearest 2μ. The symbols (α, β, γ, and V) denoting the proportions of the parts are as described in the legend to Table 1. For a description of the differences between the culture conditions, see pp. 249–250 of the text. In each of the worm broth agar cultures, the figures given are the mean of measurements on fifteen individuals, while in the case of the concentrated saline medium, the measurements were made upon twenty individuals.

tendency to produce males. These were never observed in 'starved' or standard cultures, were very occasionally seen in 'well-fed' cultures, but were quite often produced in 'cultures from isolated individuals', although even here they never formed more than 2% of the population.

(*b*) *Variations in the salinity*

Several cultures were raised in a concentrated balanced salt solution osmotically equivalent to 0·30 *M* NaCl, containing de Witte's peptone. The mean dimensions of a group of worms raised in these cultures is given in col. 5 of Table 2. It can be seen from Table 2 that increasing the salinity of the culture medium produces the following effects on the worms: (1) an increase in the proportionate thickness of the body as a whole, and (2) a decrease in the proportionate length of the tail. Undue significance should not be attached to this point because of the great difficulty in making accurate measurements of the length of the tail on account of the fineness of the tail filament.

Other more general effects produced by culturing in saline solutions with an osmotic pressure equivalent to 0·30 *M* NaCl were as follows: (1) A greatly decreased rate of reproduction. This was because a greater time was taken to attain maturity, because fewer eggs were laid by each female, because many of the eggs failed to hatch, and because most of the larvae died before attaining maturity. (2) An increased granulation of the protoplasm throughout the body. As a result, individuals were buff coloured to the naked eye, as compared with the normal light cream colour. (3) The production of a small number of individuals with deep irregular wrinkles in the cuticle.

(*c*) *Discussion of the above variations*

It will be noted that, even under the standard culture conditions, and still more in the case of the experiments whose results are summarized above, there is a considerable degree of variation in the animal. Table 2 shows that this variation is also, to a large extent, dependent upon controllable conditions of the environment. Similar variations have been noted by Peters (1927), Clapham (1930), Buckley (1931), and especially by Mackin (1936), who criticizes the use of proportions of parts in specific descriptions.

The use of measurements such as those given in Tables 1 and 2 originated with de Man (1910), and has been employed in an elaborated form by Cobb (1935) and others in the specific descriptions of nematodes. These descriptions have often been based upon single measurements of a given dimension, without reference to variation due to environmental factors. It is doubtful whether such descriptions are adequate. Thus individuals from the opposite ends of a specific range might well be mistaken for different species. Chitwood (1930) believes that this has occurred in the case of *Rhabditis elongata* Schn. and *Rh. tenuicaudata* Menzel & Stefanski.

V. THE RELATIONSHIP BETWEEN THE NEMATODES AND OTHER INHABITANTS OF THE CULTURES

(*a*) *Bacteria*

All cultures were infected with bacteria, and observations on the cultures suggested that there was a relationship between the number of bacteria to each nematode and the success of the cultures (as measured by the rate of growth of the larvae).

Thus, shortly after the beginning of the 'well-fed' cultures, the rate of growth was comparatively slow. This may have been because there were too many bacteria present per worm. Later on, when the number of worms in these cultures had increased, the individuals grew more rapidly.

This interpretation, based on the possibility that there is an optimum number of bacteria for each *Rhabditis*, agrees with the conclusion reached by Dotterweich (1938), in his investigation of the culturing of *Rh. teres* (A. Schn.).

That bacteria probably form the normal food of the species under investigation, is suggested by the work of Zimmermann (1921), and McCoy (1929), and also by the fact that bacteria could be seen entering the intestine of the animal, but were never observed leaving the anus. The nematodes congregated in those parts of the agar where the colonies of bacteria were spreading, suggesting that actively reproducing bacteria are preferred. In addition, the occasional comparative failure of a culture could sometimes be attributed

to the dominance of a new species of bacterium in the culture, which suggests that some species are not suitable as food.

(b) Protozoa

At least one unidentified flagellate protozoon occasionally inhabited the cultures. In some newly started cultures the growth of the worms seemed to be inhibited initially by the presence of an excessive number of these Protozoa. It is possible that this was due to competition for food (bacteria) between the worms and flagellates. Later on, in these cultures, the Protozoa pass from an active to a resting stage, and then the nematodes congregate in those portions of the cultures where the resting Protozoa are most abundant. An abundance of the resting Protozoa usually foreshadowed the marked success of a culture.

The resting stages of the flagellate could be distinctly seen entering the oesophagus of the nematode, but were not seen to enter the intestine, so that there is a possibility that they were broken up in passing through the second oesophageal bulb. They were never seen to pass out through the anus.

Thus it seems probable that the resting stages of the protozoon were a significant source of food for the nematode.

Summary

1. A new species of the genus *Rhabditis* is described.

2. The development of a standard method of culturing the species is outlined, and the effect on the nematode of varying the method of culturing is detailed and discussed.

3. The relationships between the nematode and the bacteria and Protozoa of the cultures are discussed.

The writer wishes to express his deep gratitude to Prof. A. D. Hobson, for his invaluable advice and assistance at all stages. Thanks are also due to King's College, the University of Durham, for financial assistance in the form of a Post-Graduate Studentship.

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THE EFFECT OF VARIATIONS IN OSMOTIC PRESSURE UPON A FREE-LIVING SOIL NEMATODE

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(With 7 Figures in the Text)

I. Introduction

It is well known that free-living nematodes are capable of withstanding environmental conditions which would be fatal to many other types of animals. This is generally assumed to be due to the insulation of the animal from its environment by virtue of the presence of an impermeable cuticle (Krogh, 1939, p. 44).

Against this, the cuticle of certain parasitic nematodes has been shown by various workers to allow aqueous exchanges between these nematodes and their surroundings (Schopfer, 1932; Panikkar & Sproston, 1941; and unpublished work by Beadle, Hobson, and the present writer). Mueller (1928) has also demonstrated that certain non-electrolytes can pass through the body wall of the pig *Ascaris*, while unpublished work by Hobson and the present writer has demonstrated that the cuticle of the same animal is permeable to a wide range of non-electrolytes.

Very little work has been performed on free-living nematodes, except for that of Abbott & Richards (1911) and Stoklasa (1933), and in both cases *Anguillula aceti* was employed as experimental material. This work cannot be considered complete from the experimental point of view, and moreover, was performed upon a nematode occupying a very unusual environment.

It was therefore considered that an investigation of the effects of variations in osmotic pressure upon a more normal type of free-living nematode would be of interest. The species chosen for investigation occurs on rotting earthworms, and is described elsewhere under the name of *Rhabditis terrestris* (Stephenson, 1942).

II. The osmotic pressure of the medium in which the nematodes were cultured

The species was cultured by a method which is described elsewhere (Stephenson, 1942), using a boiled extract of earthworms in an agar solution. Progressive concentration of this medium was found to be inseparable from successful culturing, and it was necessary to know the range of osmotic pressure to which the species was subjected.

Two methods were employed to determine this. In the first, depression of the freezing-point determinations were made with a Beckmann thermometer, while in the second, the conductivities of dilutions of the culture medium were compared with those of standard NaCl solutions. A Kohlrausch bridge method was used for the latter determinations, and was supplied with an oscillating current of frequency about 700. The results obtained are given in Table 1.

The results obtained by the depression of the freezing-point method show the total osmotic concentration, and from them it can be concluded that during the course of a

week the concentration increases from a value equivalent to about 25 mM. NaCl to one equivalent to about 35 mM. The results obtained by the conductivity method indicate the contribution which electrolytes make towards the total osmotic concentration, and show that approximately two-thirds of the depression of the freezing-point values are due to the presence of electrolytes.

In general, it may be concluded that a 30 mM. NaCl solution will be approximately isotonic with the environment in which the nematodes were cultured. It is of interest to note that the osmotic concentrations of different lots of culture media were surprisingly constant.

Table 1. *Showing the osmotic concentrations of the medium in which the* Rhabditis *were cultured. Results* (*a*) *were obtained from the same series of three cultures, a dilution of* 1/30 *being employed in the conductivity determinations. Results* (*b*) *were obtained from a series of cultures made up* 21 *months previously, and a dilution of* 1/100 *was used in the conductivity determinations.*

	Osmotically equiv. mM. of NaCl to the nearest 5 mM.	
Solution	By D.F.P. method	By conductivity method
Culture medium	(*a*) 25, 25	(*a*) 15, (*b*) 15
5-day-old culture	(*a*) 30	(*a*) 20
7-day-old culture	(*a*) 35	(*a*) 25, (*b*) 25

III. General experimental method

Female specimens which were about to begin laying eggs, and which had been reared by the standard method, were used as experimental material. They were removed from cultures on a fine glass needle and transferred to 1 c.c. of solution in a 5 cm. watch glass. The solutions employed (see below) were changed three times, whenever possible by the removal of the nematodes to a fresh solution. Observations were made microscopically, and the solutions were covered by glass plates during prolonged observation.

The effects of immersion in three kinds of solutions were investigated: (1) glass-distilled water, (2) a saline solution approximately isotonic with the culture medium (osmotically equivalent to 20 mM. NaCl), (3) saline solutions more concentrated than the culture medium. All saline solutions were made up in glass-distilled water and contained NaCl, KCl, and $CaCl_2$ in the proportion of Locke's solution, with the addition of $NaHCO_3$ to bring the *p*H to 7·2.

IV. Effects produced by immersion in media of different osmotic pressures

A large number of effects were observed, and these are discussed below under the headings of changes in the size of the body, in the appearance and behaviour of the alimentary canal, and in the movement of the body as a whole.

(1) *Changes in the size of the body*

On immersion in saline solutions isotonic with the culture medium, no differences were observed as compared with conditions in the cultures.

In glass-distilled water, on the other hand, marked increases in size were noted. These could only be measured with difficulty because of the undulating movement of the animals,

and because of their small size. Finally, the only method which could be employed under the experimental conditions was that of measuring their length changes. This was best accomplished by means of a camera lucida, since blurring of the negatives of photomicrographs commonly occurred owing to the movement of the animals in a vertical plane.

Measurements were made by twisting a fine wire along the antero-posterior axis of a camera lucida image of an individual, the original length being determined by a large number of measurements upon an individual in a thin smear of culture medium. Since somewhat large individual variations were observed, all experiments were made upon five individuals, and the mean results are given below. On Fig. 1*a* the normal swelling curve of five individuals is shown, and it will be noted that there is a rapid increase in length

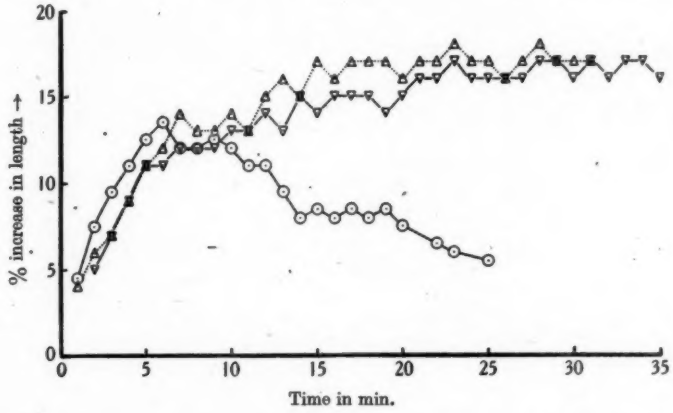

Fig. 1. Showing the mean percentage increases in length of five individuals after immersion in solutions hypotonic to the culture medium.

(*a*) Normal swelling curve on immersion in distilled water.

(*b*) Immersion in *M*/100 KCN instead of distilled water.

(*c*) Injured animals immersed in distilled water.

during the first 5 min. of immersion, to about 113% of the original length. After this there is a slower return to a more normal length, and only 5% of swelling is observed after 25 min. After this measurements had to be discontinued, owing to the rapid movement of the animals.

The effect of certain factors upon this swelling was investigated. It was shown that the temperature of culturing had no apparent effect upon the swelling curve. Slight injury, caused by exposing the worms to the atmosphere for about 1½ min., had the effect of abolishing the return to normal length, and an approximately theoretical swelling curve was obtained. Immersion in *M*/100 KCN, instead of in distilled water, produced exactly the same effects. The effects of injury and cyanide treatment are both shown on Fig. 1 with the normal swelling curve.

On immersion in hypertonic saline solutions the size of the body was reduced, and this was particularly well marked in the more concentrated solutions where it was accompanied by wrinkling of the cuticle. Measurements of this shrinkage were made by the

same method as described above, but no values could be obtained for the initial stages because the nematodes were moving too rapidly.

The results obtained are shown on Fig. 2. This figure shows that the rate and extent of the shrinkage increases with the strength of the solution employed, but that at the higher concentrations the maximum amount of shrinkage tends to be proportionately less. This, however, may in part at least, be due to the limitations of the method employed, since only lengths were measured. These probably only give a reasonably accurate

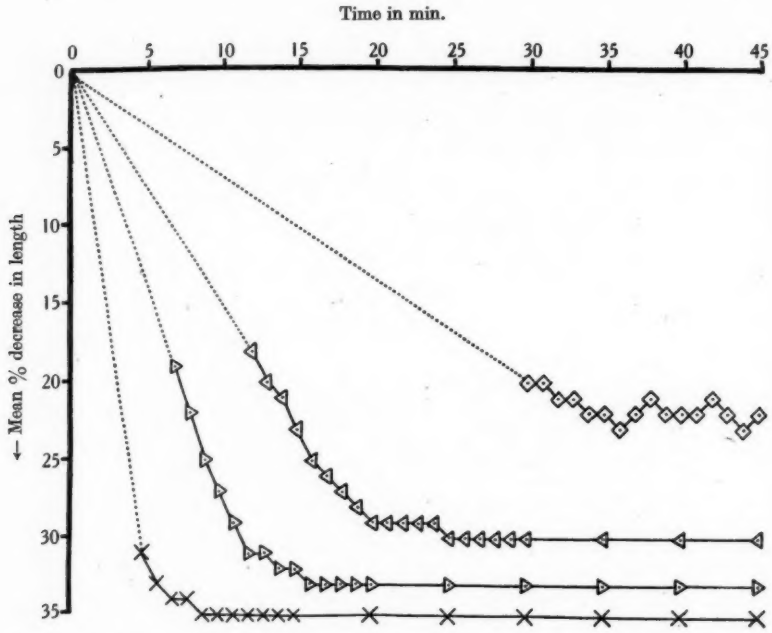

Fig. 2. Showing the mean percentage decreases in length of five individuals after immersion in balanced salt solutions hypertonic to the culture medium.

(*a*) Osmotically equivalent to 0·17 *M* NaCl.
(*b*) Osmotically equivalent to 0·34 *M* NaCl.
(*c*) Osmotically equivalent to 0·51 *M* NaCl.
(*d*) Osmotically equivalent to 0·67 *M* NaCl.

indication of the volume of the animal so long as its shape remains the same. In the above experiments this was not so, and marked wrinkling and distortion occurred in the more concentrated solutions.

After a considerable time there was a reduction in the amount of shrinkage, and the details of the process were followed in two different strengths of solutions (equivalent to 0·34 and 0·51 *M* NaCl respectively). The results are given on Fig. 3, where it will be noted that, apparently, the recovery in the more concentrated solution is more rapid and more complete than in the more dilute. Actually this was due to the fact that the animals in

the more concentrated solution died before the experiment finished, three of the batch of five being dead after 26 hr.

The marked irregularities in the later stages of recovery in the more dilute solution should be noted, and will be commented on at a later stage.

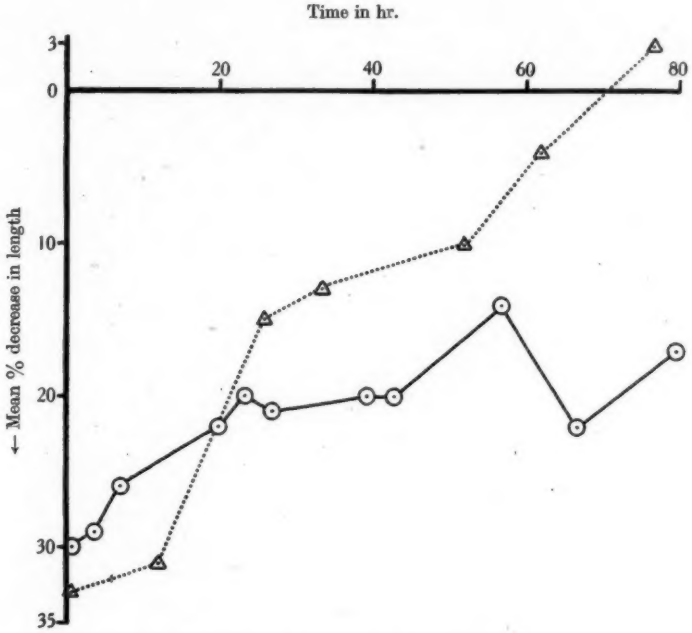

Fig. 3. Re-expansion curves after immersion in concentrated balanced salt solutions. Five individuals were used in each solution, and the lengths are expressed as mean percentages of the original length before the experiment.

Solution osmotically equivalent to 0·51 *M* NaCl.

Solution osmotically equivalent to 0·34 *M* NaCl.

(2) *Changes in the appearance and action of the alimentary canal*

In the cultures, the second oesophageal bulb pumps fluid into the intestine at a fairly regular rate, and this distends the lumen. At intervals the posterior body wall contracts, and fluid is forced through the anus to the exterior. This reduces the distension of the intestinal lumen, and the appearance of the anterior portion of the intestine alters accordingly, as shown on Fig. 4 *a*, *b*.

The removal of the fluid is accompanied by a vigorous twisting movement of the posterior part of the body, and also by a marked reduction in its length (up to 6%). Since these length fluctuations were of a considerable magnitude, it was necessary to take several measurements of length when this was required (see p. 255). These fluctuations are also of some systematic importance, since length measurements are used in free-living nematodes as specific criteria. If these vary from one time to another in the same in-

dividual, as well as from one individual to another, their continued use in specific descriptions is open to severe criticism (see Stephenson, 1942).

On immersion in the isotonic saline solution, the only change observed in the above was a reduction in the rate of entry of liquid into the intestine, presumably due to the reduced rate of oesophageal pumping.

On immersion in glass-distilled water, no oesophageal pumping occurs during the first 15 min., but in spite of this the lumen of the intestine becomes distended in a similar manner to that described above. In addition a slight pulling out of the intestine in an antero-posterior direction occurs, and this change, like all the others, is most easily seen in the anterior part of the intestine (Fig. 4*c*). As in the cultures this is followed by the expulsion of liquid through the anus to the exterior, but this does not begin until the peak of the swelling curve has been passed. On the lower portions of the swelling curve, the expulsion of fluid is almost as rapid as in the cultures, and at this stage occasional and somewhat irregular pumping of the oesophagus is observed.

On immersion in hypertonic saline solutions, the oesophagus stops pumping, and the anterior part of the intestine soon presents an appearance very similar to that observed in cultures just after the voiding of fluid. In the more concentrated solutions, this is followed by the twisting of the oesophagus and the pushing of the second oesophageal bulb into the anterior part of the intestine, as shown in Fig. 4*e*.

When recovery of size occurs in these solutions, it is accompanied by changes in the appearance of the intestine. The early stages of recovery are restricted to the anterior and posterior parts of the body, and are accompanied by a pronounced stretching out of the intestine, as shown in Fig. 4*h*. In the more concentrated solution employed in these experiments ($\equiv 0{\cdot}51\,M$ NaCl), the stretching usually proceeded to such an extent that the alimentary canal became broken, generally at the junction of the oesophagus and intestine. In the less concentrated solution employed ($\equiv 0{\cdot}34\,M$ NaCl), instead of this, the middle of the body expands, and the proportions of the body generally, including the alimentary canal, approximate to normal. The lumen of the intestine eventually presents a typically 'filled' appearance, similar to that shown on Fig. 4*a*. After about 40 hr. of immersion, intestinal fluid is removed in the normal way. This removal is accompanied by all the appearances of a return to a condition of maximum shrinkage, and the length of the body decreases from about 15% before the removal to about 25% after. Following this a second re-expansion occurs, more rapidly than the first, and the cycle is repeated several times. It was due to this cycle that the later stages of recovery in this solution appear very irregular on Fig. 3, and since it was found that the contractions were brought about by slight mechanical stimulation, such as shaking the containing vessel, observations were discontinued.

(3) *Changes in the movement of the body*

On immersion in isotonic saline solutions, the only effect observed was an increase in the rapidity of the undulating movements of the body which was typical of a reduction in the viscosity of the medium.

During the first 5 or 10 min. after immersion in distilled water, reduced spontaneous movement was observed, and in addition, only sluggish movement occurred on stimulation with a glass needle. This was followed by a recovery of normal movement which was complete in from 20 to 30 min. A great deal of variation was observed between individuals

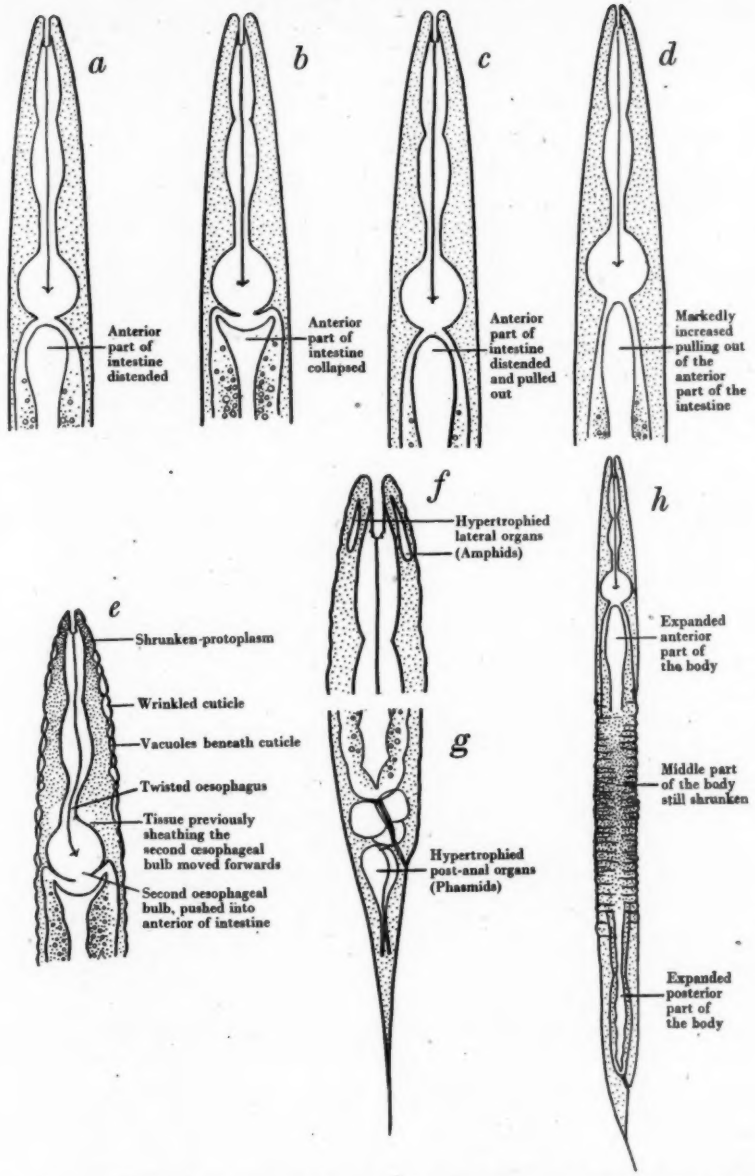

Fig. 4. (*a*) Anterior part of the intestine when distended with food. (*b*) Anterior part of the intestine after the removal of material via the anus. (*c*) Anterior part of the intestine after immersion in distilled water. (*d*) Anterior part of the intestine of an animal, imperfectly 'acclimatized' to a concentrated medium, on immersion in distilled water. (*e*) Anterior region of the body, after immersion in concentrated balanced salt solutions. (*f*) Paired lateral vacuoles formed at the anterior end of the body on immersion in concentrated media. (*g*) Paired lateral vacuoles formed at the posterior end of the body on immersion in concentrated media. (*h*) Re-expansion of the anterior and posterior ends of the body on prolonged immersion in concentrated media. (All semi-diagrammatic.)

from different cultures, and in some very little reduction in movement occurred. It was later found that this was partially dependent upon the temperature of culturing, the greatest reduction of movement being produced in animals cultured at temperatures above 17° C. For this reason all the later experiments upon the effects of distilled water were performed upon animals raised under these conditions.

After immersion in hypertonic saline solutions, typically there is initially a marked increase in the rapidity of the undulating movements of the body, best described as 'lashing movement'. This is followed by periods during which intermittent coiling of the body into and out of tight spirals is observed. This can best be described as 'spiralling movement'. Later in the more concentrated solutions, there is a decrease in spontaneous movement, which finally ceases. Finally in the strongest solutions which were employed (equivalent to 0·7 *M* NaCl) no movement can be produced even on severe stimulation with a needle. On more prolonged immersion in these solutions, the extent of the recovery of movement depended on the strength of the solution. In the two solutions employed, slight twitching movements of the extreme anterior end were first observed. In the stronger solution ($\equiv$0·51 *M* NaCl) this was followed by the loss of all movement, complete after about 25 hr. In the less strong solution ($\equiv$0·34 *M* NaCl) there was instead a return to almost normal movement in about 25 hr., after which the cycle of contractions of the body (with loss of movement) and re-expansion (with regaining of movement) occurred as previously described.

(4) *Other changes produced*

In the isotonic saline solution no other changes were observed.

In distilled water, during the rapid initial swelling, certain changes in the appearance of the protoplasm in the anterior part of the body occurred, in the region of the excretory system. Since the excretory system of the species was never seen, it cannot be stated with certainty that the changes involved this system, although this seemed probable.

In hypertonic saline solutions various other changes were noted, particularly in the appearance of the protoplasm. Occasionally, a number of vacuoles of different sizes were seen between the hypodermis and the cuticle of the nematode, as shown in Fig. 4*e*. They were observed in various parts of the body, particularly in the anterior region. Superficially they seemed comparable to the spaces which occur between the protoplast and cell wall of a plasmolyzed plant cell.

In addition to this another series of vacuoles were observed, which differed from the above in their larger size, and in the constancy of their positions. These vacuoles were paired and in a lateral position, at both the anterior and posterior ends of the body, the posterior vacuoles being observed more frequently than the anterior ones. Within limits, the longer the nematodes were left in the solutions, and the stronger the solutions, the larger the vacuoles. The posterior vacuoles were finally identified as hypertrophied 'phasmids' whose appearance in the normal animal had been overlooked up to this stage. The anterior vacuoles were probably formed from similar pre-existing structures, but since these were too small to be observed, there remains the possibility that they arise de novo. It seems likely that they are greatly hypertrophied 'amphids' or lateral organs, which Chitwood (1930) believes to be of universal occurrence in nematodes.

The anterior and posterior vacuoles are shown on Fig. 4*f* and Fig. 4*g* respectively. In addition to being observed during the process of shrinkage, they were frequently seen

during the recovery process in 0·34 *M* NaCl, more particularly during the period of most rapid expansion which occurred after 20–30 hr. of immersion.

V. CHANGES PRODUCED IN THE ABOVE EFFECTS BY ALTERATIONS IN THE OSMOTIC PRESSURE OF THE CULTURE MEDIUM

To investigate the degree of dependence of the above effects upon the osmotic pressure of the culture medium, certain experiments were repeated upon animals cultured in media of increased osmotic pressure. Two methods were used to increase this. In the first, animals were removed from the standard culture medium and treated for various times with a concentrated balanced salt solution, osmotically equivalent to 0·17 *M* NaCl. In the second, animals were cultured for different periods in the standard culture medium, to which NaCl had been added. Immediately after the removal of experimental material, the concentration of Cl′ was determined, and by comparison with values from the standard medium, the increase in concentration was determined.

Only the effects of immersion in glass-distilled water were investigated, and the results are given below.

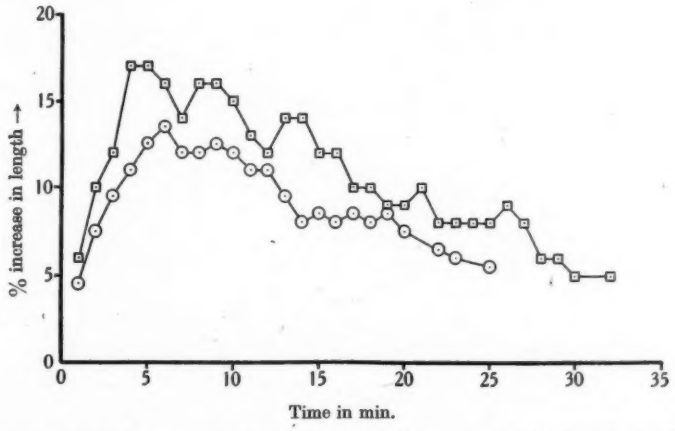

Fig. 5. Showing the effect of a comparatively short immersion in a concentrated balanced salt solution upon the swelling curves produced in distilled water.

Animals from standard cultures.

Animals immersed for *c.* 35 hr. in a balanced salt solution (≡0·17 *M* NaCl).

(1) *Changes in the size of the body* (Figs. 5–7)

Figs. 5 and 6 show that a comparatively short immersion in a concentrated balanced salt solution increases the extent and duration of the swelling produced in distilled water, but that a more prolonged immersion tends to reduce these increases towards the original amounts observed with animals from standard cultures. The same effect is shown on Fig. 7, where a large increase in the amount and duration of swelling is shown after a short immersion in a concentrated culture medium. After a more complete 'acclimatization', produced by culturing the species in this medium for three generations, the swelling caused by distilled water is considerably reduced.

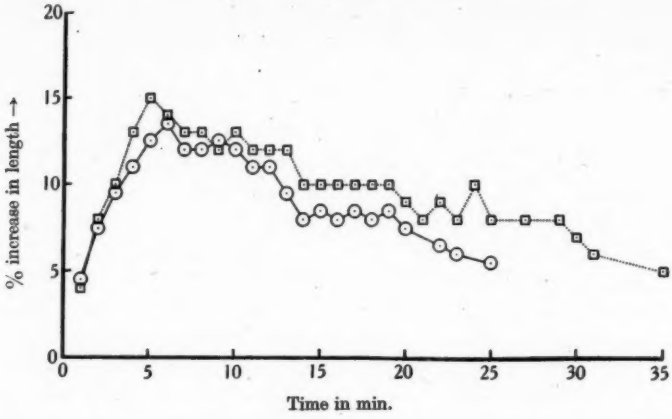

Fig. 6. Showing the effect of a comparatively long immersion in a concentrated balanced salt solution upon the swelling curves produced in distilled water.

Animals from standard cultures.

Animals previously immersed for *c.* 114 hr. in a balanced salt solution (≡0·17 *M* NaCl).

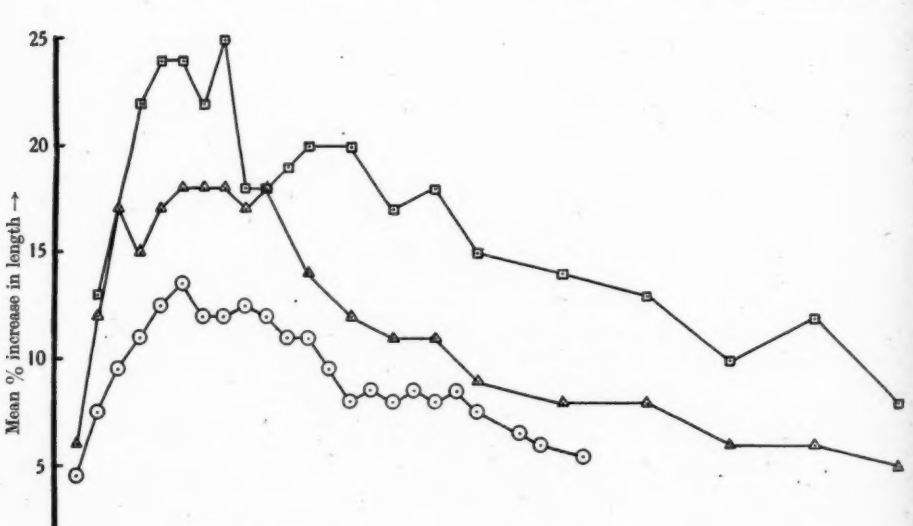

Fig. 7. Swelling curves on immersion in distilled water, showing the effect of culturing for various periods in a hypertonic medium.

Animals from standard cultures.

4 days in standard medium with NaCl added (≡0·191 *M*).

Cultured for three generations in the above medium.

(2) *Changes in the alimentary canal*

These changes were similar to those mentioned above, and after a short immersion in concentrated culture medium, the pulling out of the anterior part of the intestine produced by distilled water was markedly increased (Fig. 6*d*). A more prolonged treatment caused the amount of swelling to return nearer to that observed in animals from standard cultures.

(3) *Changes in movement*

No detailed results are available on the effect of treatment with concentrated media on the reduction of spontaneous movement which occurs in distilled water, but in general the results obtained were similar to those described above, and showed that after 'acclimatization' to concentrated solutions, the effects of immersion in distilled water were not markedly different from those obtained with animals from standard cultures.

VI. DISCUSSION

The increase in size of the body which occurs in distilled water, and the decrease in concentrated saline solutions, show that water can pass both into and out of the body under the influence of osmotic forces.

This passage may occur through the cuticular external surface of the body, through the various apertures in the cuticle, or through the alimentary canal. The stretching out of the alimentary canal in distilled water is considered to be of significance in that it suggests that the periphery of the body swells more rapidly than the gut. The latter, being attached to the body wall at the anterior and posterior ends would thus be pulled out longitudinally. The pushing of the second oesophageal bulb into the anterior of the intestine, as occurs in concentrated media, probably indicates in a similar manner that the periphery of the body has shrunk more rapidly than the alimentary canal, which can thus only fit into the reduced available space by becoming twisted. It therefore seems probable that water is exchanged more rapidly through the external surface of the body than through the lining of the alimentary canal.

Frequent attempts were made to try and identify regions on the external surface of the body where aqueous exchanges were occurring most rapidly, generally without success. In certain cases, most rapid shrinkage was definitely observed to occur at the extreme anterior end of the body when the animal was immersed in concentrated saline solutions, but these cases were not common. An example is shown on Fig. 4*e*. It therefore seems probable that the main exchange of water occurs through the external surface of the body as a whole, rather than through the apertures in the cuticle, which is apparently permeable to water in this species, as in the parasitic nematodes investigated by Schopfer (1932), Panikkar & Sproston (1941), and by Beadle, Hobson, and the present writer (unpublished).

The vacuoles which occur on the general surface of the body after immersion in concentrated media are considered to be of great importance. By analogy with the conditions in a plasmolyzed plant cell, it seems probable that the cuticle is more permeable to the substances in solution than the underlying hypodermis. It was later shown that a previous treatment with cyanide solutions produced marked decreases in the rates and amounts of swelling in distilled water, and also in the rates and amounts of shrinkage in

concentrated saline solutions. A detailed interpretation of these results will not be attempted at this stage, but it is worthy of note that these results also suggest that living tissues, rather than dead cuticle, are primarily responsible for controlling the permeability of the body wall.

On more prolonged immersion in distilled water, the swelling of the body is reduced, and there is a return of normal movement. A process of recovery is evidently occurring, and associated with it, water is being lost from the body. This recovery is inhibited by injury, or by the presence of cyanide, and its later stages are accompanied by the active removal of water through the rectum as the result of vigorous contractions of the body wall. All these observations suggest that recovery is due to the existence of an active method of osmotic regulation.

The parts of the body responsible for this could not be decided upon with certainty, because the small size of the species prevented the isolation of different portions by ligaturing or other methods. The probability that the alimentary canal plays some part in this is indicated by the periodic removal of liquid through the rectum during the later stages of recovery. The fact that a significant decrease in swelling had occurred before rectal movements were first observed suggests that there are other methods of removal. The possibility that the excretory system may be partially responsible is suggested by the alteration in the appearance of the protoplasm in the portion of the body where the tubules of this system probably occur.

The osmotic pressure of the standard culture medium is lower than that of the body fluids of most fresh-water animals (see Heilbrunn, 1937, p. 102; and Krogh (1939)), and therefore probably lower than that of *Rhabditis*. In the cultures, an endosmosis of water, and its subsequent removal against an osmotic gradient, is thus to be expected. On this interpretation, initial swelling in distilled water will be due to water entering more rapidly than it can be removed by an osmo-regulatory mechanism. Later, the rate of entry will be reduced by the decrease in the internal osmotic pressure, and by the increase in the hydrostatic pressure within the animal. A subsequent increase in the efficiency of the osmo-regulatory mechanism so that water is removed more rapidly than it enters would account for the process of recovery to a normal size.

On immersion in concentrated saline solutions, it is evident that an increase in the internal osmotic pressure of the animals must occur. This is proved by the shrinkage of the body, which also shows that this increase in the osmotic pressure is largely due to the removal of water. The presence of the vacuoles over the general body surface suggest that some penetration of osmotically active substances from the outside through the cuticle, and then possibly through the hypodermis, may occur.

During the process of recovery in these solutions, a slow reduction occurs in the amount of swelling which is observed on transfer to distilled water. This suggests that, in the recovery process, there is an elimination of osmotically active material from the body of the animal by some mechanism as yet unknown.

It is conceivable that this mechanism may be connected with the lateral organs (amphids) and the post-anal organs (phasmids), which become greatly enlarged when the animals are placed in solutions of high osmotic pressure. Since the former structures were not observed at all in the normal worms, and only with difficulty under the above conditions, this tentative suggestion as to their function must be treated with caution.

SUMMARY

1. The osmotic pressure of the medium in which the nematodes were cultured has been shown to be equivalent to that of about 30 mM. NaCl.

2. The effects of immersion in a saline solution isotonic with the above, in glass-distilled water, and in hypertonic saline solutions, have been described. Particular attention was paid to changes in the length of the body, in the appearance and behaviour of the alimentary canal, and in the movement of the body.

3. The results obtained have been discussed, and the following tentative conclusions reached:

(*a*) The main aqueous exchanges occur through the cuticle and protoplasm covering the periphery of the body, and are controlled by the protoplasm rather than by the cuticle. The cuticle thus does not act as an impermeable outer covering which insulates the animal from its environment.

(*b*) The species normally lives in an environment which is hypotonic to the body fluids, and maintains its internal osmotic pressure by means of an active method of osmotic regulation. The alimentary canal is probably partially, but not wholly, responsible for this regulation.

(*c*) Swelling occurs in distilled water because the removal of water in the initial stages is not rapid enough to cope with the extra inflow. Later the excess water is removed.

(*d*) In concentrated solutions, water is removed from the body, and the internal osmotic pressure may also be increased to some extent by the penetration of osmotically active substances to the body, through the cuticle and hypodermis of the body wall. Penetration through the hypodermis is slower than through the cuticle.

(*e*) During recovery in concentrated solutions, there is a slow removal of osmotically active material from the body. The hypertrophy of the lateral and post-anal organs, which sometimes occurs under these conditions, may be connected with this removal.

The writer wishes to express his deep gratitude to Prof. A. D. Hobson, for his invaluable advice and assistance at all stages. Thanks are also due to King's College, the University of Durham, for financial assistance in the form of a Post-Graduate Studentship.

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A NEW NAME FOR *STYPHLODORA COMPACTUM* DAWES, 1941, A CRITICISM OF THE PROPOSED NEW GENUS *PAUROPHYLLUM* BYRD, PARKER & REIBER, 1940, AND A REVISED KEY FOR THE SEPARATION OF SPECIES OF THE GENUS *STYPHLODORA* LOOSS, 1899

By BEN DAWES, D.Sc., *King's College, University of London*

(With 6 Figures in the Text)

I recently described two new trematodes belonging to the genus *Styphlodora* Looss, 1899, and propounded a key for the separation of fourteen species of the genus (Dawes, 1941). In preparing my paper, I inadvertently overlooked two recent publications by Byrd, Parker & Reiber. They appeared in different journals in 1940, one preceding the other by a matter of months, though precise dates are not available, so that the designations 1940*a* and 1940*b* are in chronological order. The reasons for clarifying this point will become evident when certain considerations have been made.

In the later paper, Byrd *et al.* proposed the erection of four new species of the genus *Styphlodora*, and by a curious coincidence one of them received the same name as one of my own, though distinct from it. As the name *S. compactum* was preoccupied when the description of my species was published, it is incumbent upon me to rename the homonym *S. compactum* Dawes, 1941, for which accordingly I now propose the name *S. compressa*.

In the same paper, Byrd *et al.* re-examined types, paratypes and other specimens belonging to several species of the genus *Styphlodora* and also put forward a key for the separation of known species. *S. dentipharyngeata* Chatterji, 1940, was not included, however, so that both this key and my own are incomplete. Before proceeding, as is fitting, to bring my own key up to date, I propose to point out a number of faults in the rival key and to make a few comments on the characters of the proposed new species, namely, *S. compactum*,* *S. aspina*, *S. floridanis* and *S. agkistrodontis*. The first named is almost certainly identical with *S. bascaniensis* Goldberger, 1911. The other three together represent fifteen trematodes which were collected from the kidneys or ureters of the snake *Agkistrodon piscivorus* (Lacépède) 50 miles east of Kissimmee, Florida, perhaps the same individual host, and they are certainly not distinct species, as will be shown.

In the earlier of the two papers, Byrd *et al.* proposed the erection of a new genus, *Paurophyllum*, for other trematodes which were found in the kidneys of snakes. The type species, *P. simplexus*, is based on specimens taken from *Lampropeltis getulus floridana*, and a second species, *P. megametricus*, is proposed for reception of others taken from *Agkistrodon piscivorus*. These two forms also are almost certainly identical, and there is considerable doubt as to the validity of the genus *Paurophyllum*. The astounding proposals of Byrd *et al.* that a small aggregate of trematodes which were collected from a rather uncommon location in a particular host species at precisely the same locality must be allocated to four distinct species, and two genera cannot be permitted to go unchallenged. Only by a most unlikely coincidence could such proposals prove to be acceptable. It is my intention to correct certain misconceptions which have resulted in

* The correct form of this name would seem to be *Styphlodora compacta*.

the erection of a number of very doubtful species for forms which can only be separated by using artifacts as valid characters. I have comments to make also on *Styphlodora magna* Byrd & Denton, 1938, *S. horridum* (Leidy, 1850) and *S. lachesidis* MacCallum, 1921, of which the last two are probably identical. My proposals regarding the invalidity of certain proposed species are based on information which is scattered throughout the work of Byrd *et al.* and might have been more appropriately compiled by these authors had they been as much concerned with the natural variability of individuals belonging to a trematode species as with the erection of new names. As a matter of convenience, I shall waive the chronological order of the two American papers under discussion and deal with the species of *Styphlodora* which are mentioned in the later paper (1940*b*) before proceeding to consider the proposed new genus *Paurophyllum* (1940*a*).

Styphlodora bascaniensis Goldberger, 1911

Synonym: Styphlodora compactum Byrd, Parker & Reiber, 1940

Goldberger described this species after studying three whole mounts, one set of sections and six preserved specimens of a trematode 3·8–4·9 mm. long which had been discovered in the liver of '*Bascanion constrictor*' in Virginia, U.S.A. Byrd *et al.* recovered nine additional specimens from the same host species in Georgia and Florida, U.S.A., and redefined the range in length as 3·7–6·3 mm. One of the specimens was discovered in the gall bladder of the host at Kissimmee, Florida, and from the same location in the same host species (possibly the same individual) at the same place they came upon a solitary specimen 3·8 mm. long, for which they proposed the erection of the species *Styphlodora compactum*.

Now the *smallest* specimens which were studied by Goldberger and by Byrd *et al.* and the specimen of *S. compactum* are of almost equal size, respective lengths being 3·8, 3·7 and 3·8 mm. In attempting to compare and contrast the three lots of trematodes we may assume, therefore, that the *lower* values of a range of measurements for various organs of *S. bascaniensis* as described by Goldberger and redetermined by Byrd *et al.* characterize the smallest specimens, though this is not specifically stated to be the case. Working on this basis, i.e. selecting size data which relate equally small specimens, we obtain some very instructive measurements (Table 1). Comparison of these data shows clearly that *S. compactum* is nothing more than a rather narrow specimen of *S. bascaniensis*, a fact which in itself partially explains the compactness and greater extent of the vitellaria, the more median position and greater overlap of the testes, and the pattern made by the uterus in front of the testes, three characters by which specific identity of the former trematode is claimed. The slightly different extent of the vitellaria is not a valid criterion of distinction, because similar variation has been observed in *S. elegans*, and the large size of the follicles in *S. compactum* (an observation which is unaccompanied by measurements) is explicable on the assumption that they are less 'spent' in this small specimen than in larger specimens of *S. bascaniensis*. Equally great differences are seen in well-authenticated specimens of the latter and may be explained in the same way, Goldberger's trematodes being the smaller ones (see Figs. 8 and 9 of Byrd *et al.*, which are reproduced as faithfully as possible in my Figs. 1 and 2). The proportionately large size of the ovary is not a valid criterion of distinction for *S. compactum* in view of the much smaller size of the testes in Goldberger's determinations. Spines are not consistently uniformly present in *S. bascaniensis*, and the lack of them in *S. compactum* does not carry

much weight in the light of general resemblances seen in respect of other characters. Neither does the different size of the egg capsules, because little can be fairly deduced from the single determinations of size which have been put forward.

For these reasons, I have no hesitation in declaring my opinion that *S. compactum* should be regarded as a synonym of *S. bascaniensis*, being no more than a small specimen of this species. Incidentally, Byrd and his colleagues claim to have brought our knowledge of *S. bascaniensis* up to date, but nevertheless they have left us with a very inadequate description of Laurer's canal in this trematode. Goldberger declared that it ends

Table 1

(Measurements in mm.)

	S. bascaniensis		
	Goldberger	Byrd *et al.*	*S. compactum*
Length of body	3·8	3·7	3·8
Breadth of body	1·7	1·72	1·55
Size of: Oral sucker	0·3 diam.	0·4 diam.	0·34 × 0·36
Ventral sucker	0·27 diam.	0·27 × 0·34	0·27 diam.
Ventral sucker to anterior end	1·13*	0·83	0·95
Prepharynx is	'Roomy'	'Short'	'Present'
Diameter of pharynx	0·105	0·157†	0·135
Length of oesophagus	0·11	0·112‡	'Short or absent'
Tips of caeca to posterior end	0·76	0·70	0·79
Anterior testis is on	Right or left	Right	Left
Dimensions of: Cirrus pouch	0·525 × 0·105	0·35 × 0·13	0·30 × (slender)
Anterior testis	0·195 diam.	0·38 × 0·54	0·25 × 0·38
Posterior testis	0·195 diam.	0·56 × 0·54	0·27 × 0·415
Ovary	Less than 0·195	0·29 × 0·29	0·27 × 0·315
Egg capsules	?	0·033 × 0·016†	0·021 × 0·015†
Extent of vitellaria	Anterior end ventral sucker to middle anterior testis	Middle ventral sucker to near posterior end posterior testis	Posterior end ventral sucker to 0·25 posterior to posterior testis
Cuticular spines	Delicate; not numerous; very sparse or absent posteriorly	Very fine; to near posterior end	Absent

* One-third body length minus half diameter ventral sucker.

† No size range given.

‡ Much shorter in Fig. 9 (my Fig. 1 B).

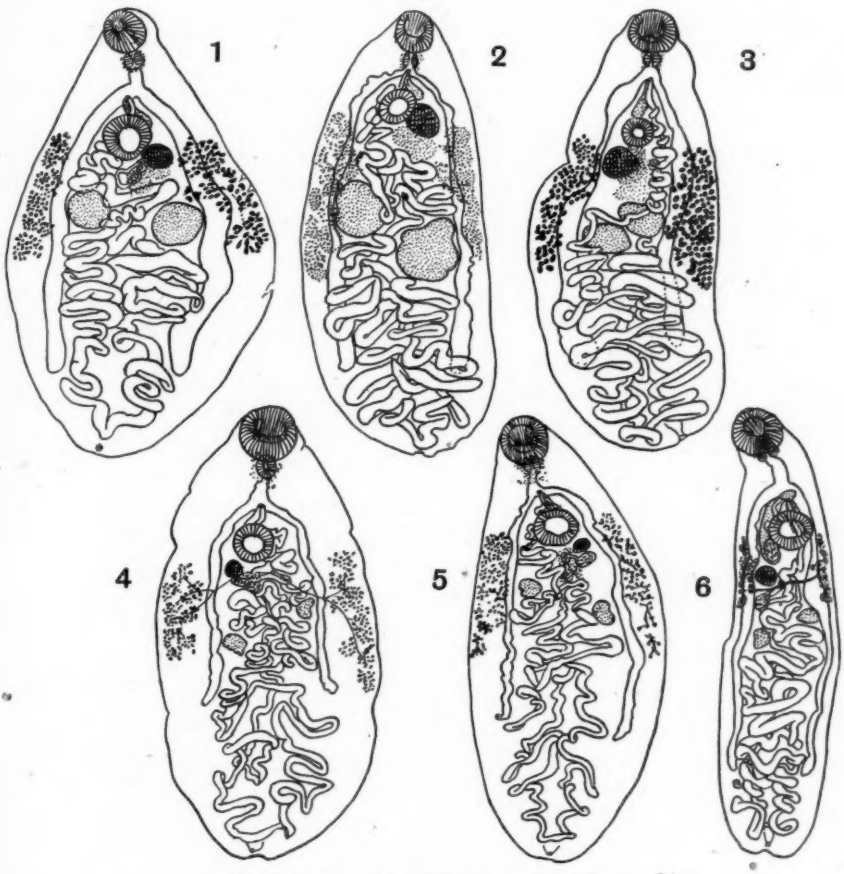
in a globular sac containing spermatozoa, sperm morulae and vitelline cells; we are now naïvely informed that it 'sometimes ends blindly'. This important character calls for detailed reinvestigation.

Styphlodora magna Byrd & Denton, 1938

Synonym: Styphlodora natricis Byrd & Denton, 1938

I have already given ample reasons for regarding *S. natricis* as a synonym of *S. magna* (Dawes, 1941). The original descriptions were based on a study of a single specimen of each kind plus an additional fragment of the former, and the host was stated by Byrd & Denton to be '*Natrix sipedon sipedon* L.' Byrd *et al.* now distinguish between the hosts of *Styphlodora magna* and *S. natricis*, naming respectively '*Natrix sipedon pleuralis* (Cope)' and '*Natrix fasciata fasciata* (Linné)'. This unqualified announcement does not affect my proposal that the two forms should be regarded as identical, which is given new weight, however, by the discovery of an additional specimen of *Styphlodora magna* in the same location (gall bladder) of a new host (*Lampropeltis getulus floridana* Blanchard) and the extension of its size range. The new specimen is 6·52 mm. long and only 0·17 mm.

longer than the specimen known as *Styphlodora natricis*, so that one character on which specific distinction was claimed for the latter, its smaller size, has disappeared. The form *S. natricis* will die hard, however, because it can be compared fairly only with an equally mutilated specimen of *S. magna*. It is distinctly possible that *S. magna* is only an overgrown and somewhat 'spent' specimen of the species *S. bascaniensis*, but scanty information hinders the formulation of a reliable opinion.



Figs. 1–6. Trematodes of the genus *Styphlodora*.

Fig. 1. *S. bascaniensis* (type material); Fig. 2. *S. bascaniensis* (new material acquired by Byrd *et al.*); Fig. 3. *S. compactum* (now *S. bascaniensis*); Fig. 4. *S. floridanis*; Fig. 5. *S. agkistrodontis* (now *S. floridanis*); Fig. 6. *S. aspina* (now *S. simplexa*). All in ventral view, after Byrd *et al.*

Styphlodora aspina Byrd, Parker & Reiber, 1940

It is proposed to erect this species for four specimens and a fragment of a fifth which were found in the kidney of *Agkistrodon piscivorus* (Lacépède) 50 miles east of Kissimmee, Florida. I shall defer consideration of its characters and any pronouncement on its validity or invalidity until the genus *Paurophyllum* has received some criticism.

Styphlodora floridanis Byrd, Parker & Reiber, 1940

Synonym: Styphlodora agkistrodontis Byrd, Parker & Reiber, 1940

The erection of a species, *S. floridanis*, is proposed to accommodate four trematodes which were recovered from the ureters of *Agkistrodon piscivorus* 50 miles east of Kissimmee, Florida, and the erection of another species, *Styphlodora agkistrodontis*, is proposed for six specimens from the same location in the same host species in precisely the same locality. Possibly all the trematodes were obtained from the same individual host, because there is no information to the contrary. Whether or not this is so, there is no doubt that the ten trematodes belong to one and the same species, as the data in Table 2 clearly show. The obvious overlap in the dimensions of organs and parts completely justify the conclusion of identity, and it would be remarkable if all the individuals of the best authenticated species of trematode agreed more closely than do these two sets

Table 2

(Measurements in mm.; means bracketed)

	S. floridanis	*S. agkistrodontis*
Length of body	2·52–4·41 (3·85)	3·5–4·82 (4·30)
Breadth of body	1·21–2·13 (1·74)	1·26–2·25 (1·8)
Dimensions of: Oral sucker	0·35–0·496 (0·44)	(0·41 × 0·43)
Ventral sucker	(0·31 × 0·35)	(0·32 × 0·36)
Ventral sucker to anterior extremity	0·77–1·17 (0·94)*	0·78–1·14 (1·20)
Length of prepharynx	0·13†	0·085†
Dimensions of pharynx	(0·14 × 0·15)	(0·12 diam.)
Length of oesophagus	0·13–0·22 (?)	0·137–0·27 (0·20)
Tips of caeca to posterior extremity	0·70–1·40 (1·14)	1·10†
Dimensions of cirrus pouch	(0·36) × 0·082‡ (?)	(0·45) × 0·07–0·095‡ (?)
Anterior testis is on	Left 'usually'	Right
Dimensions of: Anterior testis	(0·19 × 0·18)	(0·19 diam.)
Posterior testis	(0·18 diam.)	(0·22 diam.)
Ovary	(0·13 diam.)	(0·13 diam.)
Egg capsules	0·052–0·069 × 0·026–0·034 (?)	0·060 × 0·034† (?)

* Obvious misprint (1·17 μ) in figure here.

† No size range given.

‡ Clear overlap in the detailed figures.

(?) No mean figures stated.

of flukes. Characters other than those mentioned in the table also agree significantly. The cuticle is without spines, 'gland cells' encircle the pharynx, and the genital pore is said to lie 'over' the bifurcation of the 'caeca' in both sets of specimens. The excretory systems are said to be identical. The metraterm is 'very weakly muscular' in one and 'weakly developed' in the other, but 'about one-half length of cirrus sac' in both. The egg capsules are said to be 'fully embryonated when oviposited' in both lots of specimens. In response to the claim that *S. floridanis* and *S. agkistrodontis* can be separated by the larger body size, longer caeca and larger internal structures in the latter, I would point to the figures, which speak for themselves. In case differences in the length of the caeca as shown in Figs. 15 and 16 (reproduced as faithfully as possible in my Figs. 4 and 5) be regarded as consistent ones, I would again draw attention to the range stated for *S. floridanis* in respect of this character§ (a range is not given for *S. agkistrodontis*), remarking on the close similarity between the means.§ The diagrams of Byrd *et al.* surely

§ Strictly, the mean distance between the tips of the caeca and the posterior extremity in *S. floridanis* and the corresponding single measurement for *S. agkistrodontis*.

represent extreme differences between individual trematodes of the two lots! Thus, the apparent difference in the extent of the vitellaria is probably unreal; certainly, this character cannot be used to determine specific identity when consistently compared with organs other than the caeca. Finally, it is to be deplored that the host genus should be named four times on pp. 319 and 320, each time with a different spelling (*Agistrodon*, *Agkistrodon*, *Akistrondon* and *Agkistrondon*).

Styphlodora horridum (Leidy, 1850)

Synonym: Styphlodora lachesidis MacCallum, 1921

Byrd *et al.* re-examined the type specimen of *S. horridum*, and their measurements, which differ slightly from Leidy's, show the breadth of the body to be 0·174 the length. From Leidy's fine diagram I deduced that it could not be more than 0·17, though his measurements erroneously indicated it to be as much as 0·28 (Dawes, 1941). The Americans also re-examined *S. lachesidis* and they brought to light without comment some remarkable data, which would no doubt have been even more striking if the twenty-six specimens of *S. horridum* which Leidy mentioned had received closer attention. It seems that only a single specimen was examined, because the only size range set down refers to the egg capsules. The original and revised descriptions of *S. lachesidis* differ considerably. MacCallum gave the length of the oesophagus as five times that of the prepharynx, the size of the ventral sucker as twice that of the oral, characters which I used in my key for want of more precise ones. Byrd *et al.* state that the oesophagus is 1–5 times as long as the 'pharynx' and that the ventral sucker is less than half as large again as the oral. It happens in consequence that *S. horridum* and *S. lachesidis* show much closer agreement than original descriptions suggest. In fact, the single measurements of the organs of the former come within the size ranges quoted for the latter in many important respects: in regard to the length and breadth of the body, the length of the oral and ventral suckers and the distance which separates the latter from the anterior extremity, the dimensions of the pharynx, the length of the oesophagus and of the cirrus pouch. The egg capsules of *S. lachesidis* are slightly the larger, but there is considerable overlap of the ranges given, and this is increased if MacCallum's determinations are accepted. Other characters showing close resemblance in the two forms include the vitellaria, which are of similar extent, spread consistently over the ventral surfaces of the caeca and are represented by 20 or by 23–25 follicles on either side of the body. The more anterior testis is the left one, and the genital pore is situated to the left of the median plane, the ovary to the right of it, in each case. Differences exist, the most marked being the slightly smaller testes of *S. horridum* and the spineless cuticle of *S. lachesidis*, neither of which characters carries much weight in establishing identity. According to the diagrams of Byrd *et al.* (Figs. 2, 6), *S. lachesidis* has a more attenuated anterior extremity, but there is ample evidence that the specimen of *S. horridum* shown is much contracted anteriorly, though the oesophagus, contracted as it is, measures 0·22 mm. in length and is thus twice as long as the pharynx (0·113 mm. long). This is sufficient to account for the slightly more forward positions of the testes. The identity of *S. lachesidis* with *S. horridum* can easily be settled by re-examination of the original specimens to which Leidy referred, and for this reason it is to be regretted that Byrd and his colleagues were content to examine only a solitary specimen.

THE PROPOSED GENUS *PAUROPHYLLUM* BYRD, PARKER & REIBER, 1940*a*

We might first of all consider the proposal of Byrd *et al.* to erect the species *Paurophyllum simplexus* and *P. megametricus* already mentioned. The former is said to be distinguishable from the latter by its smaller size, the larger size of its testes and ovary, the proportionately smaller cirrus pouch, the smaller and less muscular metraterm, the uncoiled condition of the uterus in front of the testes, the regularity of the caeca, the larger and more uniformly distributed vitellaria and the larger 'ova'. Some of these statements simply do not fit the facts, if we accept the range of measurements as such, and others are based on artifacts. Comparison of the data assembled in Table 3 indicates clear overlap in respect of the ranges* of the length and breadth of the body, the size of the oral and ventral suckers, and the distance of the latter from the anterior extremity, the size of the pharynx, oesophagus, and cirrus pouch and the dimensions of the gonads and the egg capsules. Most assuredly, if the two lots of trematodes were shuffled, the

Table 3

(Measurements in mm.)

	Paurophyllum simplexus	*Paurophyllum megametricus*
Length of body	1·58–2·24	1·20–1·85
Breadth	0·50–0·67	0·51–0·80
Oral sucker	0·16–0·22 × 0·17–0·22	0·13–0·23 × 0·17–0·21
Ventral sucker	0·16–0·22 × 0·17–0·22	0·15–0·22 diam.
Ventral sucker to anterior end	0·44–0·76	0·39–0·53
Length of cirrus sac	0·26–0·40	0·21–0·36
Length of metraterm	0·26–0·33	0·18–0·33
Anterior testis	0·17–0·28 × 0·16–0·21	0·07–0·17 × 0·06–0·14
Posterior testis	0·15–0·30 × 0·15–0·22	0·06–0·17 × 0·03–0·16
Ovary	0·10–0·17 × 0·09–0·14	0·05–0·09 diam.
Egg capsules	0·042–0·061 × 0·023–0·030	0·043† × 0·017–0·026
Caeca to posterior end	0·23–0·35	0·15–0·35
Pharynx (diam.)	0·087–0·104	0·07–0·10
Length of oesophagus	0·087–0·20	0·052–0·095

† No size range given.

task of separating them into the original lots would be impossible without resorting to a selection of specimens in which one testis or both testes happen to be of rather small size. This character is at best an unreliable criterion of distinction, as my previous work on *Styphlodora compressa* has shown, and it is safe to say in this instance that only the hosts are distinctive. The form which is referred to as *Paurophyllum megametricus* (whose very name is misleading) is obviously nothing more than a relatively contracted form of *P. simplexus*, contraction being most evident in the breadth of the body but almost equally in the condition of the caeca, vitellaria, uterus and other organs. For these reasons I affirm categorically that *P. megametricus* should be regarded as a synonym of *P. simplexus*.

The genus *Paurophyllum* is said to differ from *Styphlodora* in certain details of the excretory system. The general terms of the diagnoses are so similar as to be interchangeable, where they are of common type. The flame-cell formula in both instances is of the 2 [(3+3+3)+(3+3+3)] or 2×6×3 type, and the sole character on which generic distinction is claimed concerns the excretory bladder. In *Paurophyllum* it is said to be

* The *ranges* in size are obviously of much greater importance than the mean values, which may be grossly misleading.

smooth and tubular, without cornua (?); in *Styphlodora* it consists of a long stem with short lateral 'outgrowths' and short cornua. The only species of the latter genus for which details have been given (Byrd *et al.* 1940*b*) is *S. agkistrodontis*, though the excretory system is declared to be identical with this in *S. aspina*, *S. compactum* (now *S. bascaniensis*) and *S. floridanis*, no details being given. This conclusion appears to have been extended to include other species of the genus *Styphlodora* as well, before data have been drawn to warrant so doing. Members of the genus differ considerably in size (as far as they are known), and it is not a foregone conclusion that the excretory system of small and large individuals of even the same species will have the same type of vesicle. Useful as is the excretory system of Trematoda in taxonomy, it is well known that the excretory vesicle may present a variety of structure from the metacercarial stage in development onwards. Thus, the condition of a 'network' develops in *Fasciola hepatica* out of a much simpler Y-shape. It is possible that in closely related forms like *Styphlodora agkistrodontis* and *Paurophyllum simplexus*, where the size of the body is said to differ appreciably, such differences as have been noted by Byrd *et al.* may represent stages in the development of one and the same type of excretory vesicle. Outgrowths of the vesicle may turn out to be more common in large individuals belonging to species of *Styphlodora* than the proposed generic distinctions allow for. Furthermore, it is coming to be more generally admitted that there is some possibility of error being incurred in diagnosis if implicit faith is placed solely in the characters of the excretory system, which should be taken in conjunction with other systems. In the present discussion, the main point at issue, the validity or invalidity of the proposed genus *Paurophyllum*, can be decided by making the following considerations. Slight differences in the form of the excretory vesicle have been shown to exist as between *Paurophyllum simplexus* and *Styphlodora agkistrodontis*, but these have not been proven to be of generic importance, because the nature of this character has not been sufficiently investigated either in different species of *Styphlodora* or in individuals of different size in any one species. The possibility of transition between the slightly different kinds of excretory vesicle during growth, therefore, has not been ruled out. On the other hand, close resemblance between the general characters of the genus *Styphlodora* and the proposed new genus *Paurophyllum* strongly suggest generic identity. For these reasons it is now suggested that *Paurophyllum* be regarded as a synonym of *Styphlodora*. If this proposal is concurred, the form *Paurophyllum simplexus* becomes *Styphlodora simplexa* (Byrd, Parker & Reiber, 1940) n.comb. (Synonyms: *Paurophyllum simplexus* Byrd, Parker & Reiber, 1940*a* and *Paurophyllum megametricus* Byrd, Parker & Reiber, 1940*a*.)

The question still remains, is *Styphlodora simplexa* a genuine new species of its genus or merely a new form of an old one? Bearing in mind the identity of host species, location in the host and locality, as well as the almost identical size range, we may compare it (taking care to include in our considerations the synonymous form) with *S. aspina*. Data which have been assembled in Table 4 show clear overlap of the ranges in size for various organs and parts and strongly suggest that we are dealing with one and the same species. The resemblances between the two forms are certainly too close to be accidental, but in fairness two differences must be noted: the lack of spines in the cuticle and the lack of a prepharynx in *S. aspina*. Both of these negative characters carry little weight in diagnosis, however, because spines are well known to be easily lost, and even Byrd *et al.* claim the presence of a prepharynx to be one of the characters of the genus *Styphlodora*.

Furthermore, there are genuine species of *Styphlodora* in which the prepharynx is absent or, at any rate, has not been seen.* The issue is in very little doubt, and I have no hesitation in affirming that the two forms should be considered identical. As *S. simplexa* arose in advance of *S. aspina* by some months, the correct name of the trematode in question is:

Styphlodora simplexa (Byrd, Parker & Reiber, 1940*a*) n.comb.
Synonyms: *Paurophyllum simplexus* Byrd, Parker & Reiber, 1940*a*.
P. megametricus Byrd, Parker & Reiber, 1940*a*.
Styphlodora aspina Byrd, Parker & Reiber, 1940*b*.

Unfortunately, the confusion to which the work of Byrd *et al.* has given rise does not end here. From the data assembled in Table 4 it seems fairly clear that while *S. simplexa* as represented by '*simplexus*', and '*megametricus*' is in some respects intermediate between the synonymous form known as *S. aspina* and *S. floridanis* (synonym: *S. agkistrodontis*), a fair comparison cannot be made because of size inequalities. One important

Table 4

(Measurements in mm.)

	S. floridanis (Syn. *S. agkistrodontis*)	*S. aspina*	*S. simplexa* (Syn. *Paurophyllum simplexus* *P. megametricus*)
Length of body	2·52–4·82	1·48–1·71	1·20–2·24
Breadth	1·21–2·25	0·35–0·42	0·50–0·80
Oral sucker	0·35–0·50 diam.	0·14–0·17 × 0·19	0·13–0·23 × 0·17–0·22
Ventral sucker	0·31–0·36 diam.	0·125–0·14 diam.	0·16–0·22 × 0·17–0·22
Ventral sucker to anterior end	0·77–1·14	0·44	0·39–0·76
Length of cirrus sac	0·26–0·50	0·25–0·27	0·21–0·40
Length of metraterm	?	?	0·13–0·33
Anterior testis	0·17–0·21 × 0·11–0·21	0·06–0·07 × 0·04	0·07–0·28 × 0·06–0·21
Posterior testis	0·14–0·25 diam.	0·08–0·10 × 0·06–0·08	0·06–0·30 × 0·03–0·22
Ovary	0·10–0·15 diam.	0·06–0·075 diam.	0·05–0·17 × 0·09–0·14
Egg capsules	0·052–0·069 × 0·026–0·034	0·042 × 0·021†	0·042–0·061 × 0·017–0·030
Caeca to posterior end	0·70–1·40	0·27–0·46	0·15–0·35

† No size range given.

consideration can be made, however. In *S. floridanis* and *S. aspina* the oral sucker is larger than the ventral one, while in *S. simplexa* the reverse is the case. The latter statement is true, however, only as far as the synonymous form known as '*megametricus*' is concerned, the form which was described as *Paurophyllum simplexus* having oral and ventral suckers of absolutely equal size (Table 3). Of the two forms the latter is the larger, which suggests that differences in the relative sizes of the suckers are influenced by the processes of growth of the trematode. Relative diminution in size of the ventral sucker during growth occurs in *Styphlodora elegans*; from being almost twice the diameter of the oral sucker, it gradually becomes less than one-third as large again (Dawes, 1941). It seems that in the species now in question a similar growth phenomenon occurs, but comparative equality of size in the smaller trematodes leads to the development of a ventral sucker which eventually becomes absolutely smaller than the oral one. Byrd *et al.* had a splendid opportunity of ascertaining whether or not this is really the case when they discovered their trematode parasites of the kidney of *Agkistrodon piscivorus* but preferred to conclude that differences in the proportions of trematodes of different

* Possibly the prepharynx was overlooked in *S. aspina*. It is short, apparently absent, in many individuals of *S. elegans*.

size necessarily imply specific identity. Plainly, they made no allowance for growth changes, normal variability of proportions in an animal which cannot be said to have a fixed shape, or even artifacts due to the perpetuation of unusual postures in fixation. How they succeeded in separating trematodes from a particular location, host and locality into four lots, calling each the basis of a new species, is difficult to imagine. What is clear, however, is the close relationship which exists between these trematodes. The question of the validity of *Styphlodora simplexa* (and its synonyms) and *S. floridanis* (and its synonym) cannot be categorically settled from the available information about them. Accordingly, it is not proposed immediately to regard them as identical, and separate places have been found for them in my key. But, at the same time, my opinion is that when the trematode parasites of the kidney of *Agkistrodon piscivorus* have been studied in sufficient detail, the latter form will merge imperceptibly into the former in the ultimate analysis and will come to be regarded as synonymous with it.

Turning now to the key which Byrd *et al.* provided for the separation of species of the genus *Styphlodora*, we find that some of the characters which were used with misleading effect by Bhalerao (1936) have been used with even greater freedom, and resultant error. Thus, *S. similis* and *S. persimilis* are separated from other species on account of their possessing deeply notched or lobed testes, though Nicoll (1914) did not mention such a character for the latter species. Going even further, Byrd *et al.* refer in their key to a notched or lobed ovary in *S. persimilis*, though Nicoll specified the shape as globular. Other discrepancies occur in the key. *S. nicolli* is separated from *S. condita* partly because of an 'elongate' pharynx, which in the text is said to measure 0·16 mm. in diameter and which, according to Bhalerao, measures 0·16–0·18 × 0·16–0·165 mm. The attributes of the testes by which *S. serrata* and *S. renalis* are separated in the key also depart from the original descriptions.

It is perhaps needless to test the key of Byrd *et al.* further, but it must be made clear that it is faulty from the first step onwards. Right away, *S. najae* and three other species are separated from *S. condita* and all other species by the postulation of differences in the extent of the vitellaria. Yet Nicoll (1912) defined the extent of these organs in *S. najae* as 'from the posterior border of the ventral sucker to the anterior border of the left (anterior) testis', while De Faria (1911) did the same for *S. condita* using the terms 'vom Vorderrande des ersten Testikels bis zum Hinderrande des Bauchsaugnapfes'. In criticizing Bhalerao for the same error, I thought it unlikely that any other systematist would discover a significant difference between what is implied in these two statements, but Byrd and his colleagues have done so, thus vitiating the first and most important step of their key. It would show not only immodesty but also lack of appreciation of the difficulty met with in defining species of Trematoda to claim that my own key is infallible. But, at least, it is as consistent with original descriptions as it can be made and it has now been modified to suit not only the findings of Byrd *et al.* but also the inferences which I have drawn from their work.

A revised key to species of the genus *Styphlodora* Looss, 1899

(1) Body oval or broadly lanceolate, maximum breadth generally more than one-third body length. Right and left testes generally separated by a wide median space.

(A) Oral sucker larger than ventral sucker.* Genital pore at or very near the bifurcation of the intestine.

(*a*) Body very broad (breadth generally greater than 40% length).

(*aa*) Laurer's canal ends in a blind sac. Egg capsules significantly smaller than 0·050 × 0·020 mm. **bascaniensis**

(*bb*) Laurer's canal does not end in a blind sac. Egg capsules significantly larger than 0·050 × 0·020 mm. **floridanis**

(*b*) Body not very broad (breadth generally less than 40% length).

(*aa*) Ventral sucker significantly less than 0·25 body length from anterior extremity. Egg capsules small (0·026–0·036 mm. long). **magna**

(*bb*) Ventral sucker significantly more than 0·25 body length from anterior extremity. Egg capsules not small (0·042–0·061 mm. long). **simplexa**

(B) Oral sucker not larger than ventral sucker. Genital pore well behind the bifurcation of the intestine.

(*a*) Maximum breadth of body more than 35% length. Prepharynx absent. Oesophagus short, practically absent. Cuticle spinous. **renalis**

(*b*) Maximum breadth of body less than 35% length. Prepharynx present. Oesophagus clearly present. Cuticle spineless. **similis**

(2) Body narrowly lanceolate, maximum breadth generally less than one-third body length. Right and left testes generally not separated by a wide, median space.

(A) Maximum breadth of body generally more than one-quarter length.

(*a*) Anterior end of body (to ventral sucker) relatively long. Bifurcation of intestine well in front of ventral sucker. Egg capsules mostly larger than 0·042 × 0·021 mm. **serrata**

(*b*) Anterior end of body (to ventral sucker) relatively short. Bifurcation of intestine immediately in front of ventral sucker. Egg capsules mostly smaller than 0·042 × 0·021 mm.

(*aa*) Suckers transversely oval. Ratio of diameters of oral and ventral suckers 5/6, always greater than 4/5, never greater than 6/7. Prepharynx absent. **persimilis**

(*bb*) Suckers rounded. Ratio of diameters of oral and ventral suckers greater than 6/7. Prepharynx present. **nicolli**

(B) Maximum breadth of body generally not more than one-quarter length.

(AA) Oral sucker significantly larger than ventral sucker. **compacta**

(BB) Oral sucker not significantly larger than ventral sucker.

(*a*) Body thin and cylindrical or narrow and flat. Suckers of approximately equal size. Genital pore well behind bifurcation of intestine.

(*aa*) Ventral sucker spinous, pharynx denticulate. **dentipharyngeata**

(*bb*) Ventral sucker aspinous, pharynx not denticulate.

(*aaa*) Caeca long (to final tenth of body). Suckers large (about 10% body length). Uterus ill developed. **najae**

(*bbb*) Caeca not especially long (to final fifth of body only). Suckers small (much less than 10% body length). Uterus very well developed. **horridum**

(*b*) Body with lateral swelling opposite vitellaria, sharply attenuated anteriorly. Oral sucker smaller than ventral (ratio of diameters 3/4). Genital pore well behind bifurcation of intestine. **compressa**

(*c*) Body tapering anteriorly from its posterior half, posterior end rounded or truncated. Oral sucker smaller than ventral (ratio of diameters 0·6–0·9). Genital pore near bifurcation of intestine.

* *S. simplexa* has suckers of absolutely equal size but when synonymous forms are taken into consideration and extreme ranges in size are noted, this statement holds good.

(*aa*) Both ends of body truncated. Ratio of diameters of oral and ventral suckers greater than 0·8. Oral sucker broader than 8% body length. Testes of irregular shape, overlapping median plane. Egg capsules shorter than 0·045 mm. **condita**

(*bb*) Anterior end of body pointed, posterior end rounded. Ratio of diameters of oral and ventral suckers less than 0·8. Oral sucker narrower than 8% body length. Testes ovoid, one on either side of median plane. Egg capsules longer than 0·045 mm. **elegans**

Note. Host, location of trematode in body of host, and locality have been left out of account, though they also serve in the separation of species.

SUMMARY

1. The homonym *Styphlodora compactum* Dawes, 1941, has been renamed *S. compressa*.

2. Several suggestions have been made regarding the invalidity of proposed species of the genus *Styphlodora*. *S. compactum* Byrd, Parker & Reiber, 1940, should be regarded as a synonym of *S. bascaniensis* Goldberger, 1911, *S. natricis* as a synonym of *S. magna* Byrd & Denton, 1938, *S. agkistrodontis* as a synonym of *S. floridanis* Byrd, Parker & Reiber, 1940, *S. lachesidis* as a synonym of *S. horridum* (Leidy, 1850).

3. A suggestion has been made to relegate the proposed genus *Paurophyllum* Byrd, Parker & Reiber, 1940, to synonymity with the genus *Styphlodora* Looss, 1899. The proposed new species *Paurophyllum simplexus* and *P. megametricus* Byrd, Parker & Reiber, 1940, are identical and should be known as *Styphlodora simplexa* (Byrd, Parker & Reiber, 1940*a*) n.comb. *S. aspina* Byrd, Parker & Reiber, 1940, is a synonym of this species.

4. A revised key to the sixteen apparently valid species of the genus *Styphlodora* has been constructed. *S. floridanis* and *S. simplexa* have been found places in it provisionally, though it is suggested that further study of the trematode parasites of the kidney of *Agkistrodon piscivorus* might result in the merging of these species into a single species, namely, *Styphlodora simplexa* (Byrd, Parker & Reiber, 1940).

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Other references are to be found in the last-mentioned publication.

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TURKEY MALARIA

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A turkey brought to this laboratory for examination in 1934 was found to be harbouring protozoal parasites in the red blood cells, but the parasite was not definitely identified at the time. The parasite was determined as a *Plasmodium* species by Mr R. Daubney when he returned from leave; and when Dr Carlton Herman was a guest worker at this Institute in 1939 Mr Daubney suggested that he might like to investigate the incidence of this particular parasite in turkeys in the neighbourhood of Kabete. A visit was then made to a nearby turkey farm, and blood smears taken from many poults. One of these birds proved to be infected with a *Plasmodium*. Using the blood of this bird, experimental infection was set up in other young turkeys.

It is not intended to give a description of the parasite and its life history, since this is being done by Dr Herman, but merely to give an account of the disease as it occurs in naturally and artificially infected birds. This disease has been found altogether in three Kenya flocks.

Ngong. This very large flock was the one visited by Dr Herman. Although the parasite was found to be infecting a young bird, there was no history of past losses which suggested deaths from malaria. Many post-mortem examinations have since been made on birds from this farm which have died from other causes, but no evidence of plasmodial infection has been noted other than the presence of pigment in certain organs; this is a feature that is discussed later in this paper.

Nairobi. A pedigree cock was brought to the laboratory, suffering from general weakness, oedema of the legs, and high blood pressure. Blood smears showed *Plasmodia*. The bird died after exhibiting cerebral symptoms. Examination of the six hens in the same run proved these to be healthy.

Songhor. A report was received that young turkeys died suddenly and adults did not do well. Mr Daubney, the Director of Veterinary Services, found that the cock bird on this farm was ill. The symptoms were persistent resting of the head on the ground, high blood pressure and oedema of the legs. This turkey, which died 3 days after arriving at the laboratory, showed numerous parasites in the blood during life.

Experimental infection of turkeys

It was difficult to obtain turkeys for experimental work and those which were procured came from areas which could not be guaranteed free from this infection. Whenever possible young turkeys, 2–6 months old, were used and the birds were repeatedly examined for parasites over a period of several days before they were used experimentally. It was found that the parasite could be easily transmitted by the inoculation into susceptible birds of 0·25 c.c. of fresh infective blood. The parasites then appeared in the blood 5–18 days after the injection.

Although careful temperature records were normally kept of all the turkeys under experiment there was no evidence that infection or the disease was associated with any

rise in body temperature which could be attributed regularly to the malarial infection. Young birds under a year old developed an acute, often fatal, disease. Twelve out of fourteen young poults infected, succumbed. Older birds appeared little affected, but as it was not possible to prove that these adults had not previously been through a natural attack, little value can be placed on work with full-grown turkeys obtained locally.

Table 1 records serial transmissions in young birds:

Table 1

Turkey no.	Donor	No. of days after inoculation when parasites seen in blood	Survival in days	Passage
87	—	9	25	3
387	—	13	24	3
2387	87	18	17	4
1929	87	13	18	4
362	2387	12	35	5
100	362	5	15	6
99	362	5	13	6
97	99	9	Survived	7
96	97	15	57	8
95	96	n.p.s.	6*	9
94	95	18	27	10

n.p.s. = no parasites seen.

* Bird no. 95 was pecked to death before it had time to show parasites, but subinoculation of its blood proved that it harboured *Plasmodia*.

SYMPTOMS

Young inoculated birds showed malaise and ruffled feathers. It was usual to find a bird dead without any acute symptoms. One poult, no. 97, was being inspected when suddenly it placed its head on the ground for a few seconds then made a complete somersault over its head. This was repeated twice, then the bird lay on its side, flapping its wings violently. It died in a few minutes. As has been stated, the two adult naturally infected birds showed brain symptoms, oedema of the legs, emaciation, and high blood pressure.

POST-MORTEM FINDINGS

Acute cases

The experimentally infected birds usually died of the acute disease and hence were in good bodily condition.

Blood. The blood invariably showed many parasites. Dr Herman will describe these forms.

Liver. Naked-eye examination showed this organ to be always dark and congested. In sections the congestion was seen to be grossly disorganizing the liver cells. Early fibroblastic changes soon set in, and if the bird survived the infection long enough, general fibrosis resulted. On two occasions there were present darkened areas covering a few square centimetres each, scattered throughout the surfaces of the livers. These proved to be early necrotic lesions, and the necrosis was obviously due to pressure and distortion created by the increased quantity of blood that the organ contained. In a few cases schizonts were seen in the endothelial cells of the small vessels. A golden-green pigment was present in the interlobular tissues.

Spleen. This organ was always dark, firm and engorged. Microscopic examination revealed a noteworthy amount of pigment. Schizonts were sometimes seen in the endothelial cells lining the small vessels. In less acute cases, this organ had an excess of connective tissue.

Kidneys. The kidneys were always dark and full of blood. Histological study revealed that acute cases had severe engorgement, especially in and around the glomeruli. In cases that had survived for longer periods the glomeruli were compressed, and later still fibrosis was evident, leading to further compression and consequential atrophy of the glomeruli and the cells lining the excretory tubules.

Lungs. The lungs were slightly oedematous. Pigment was sometimes present and schizonts were rarely seen in the endothelium of the capillary vessels.

Heart. The pericardial sac contained an excess of clear fluid.

Alimentary tract. The outstanding naked-eye changes were in the duodenal loop where this is attached to the pancreas. The lumen of this portion was of a light chocolate colour.

Scrapings of the mucosa of the above-described area revealed on microscopic examination that the villi were heavily laden with pigment. Sections clearly demonstrated the intracellular pigment associated with engorgement of the vessels.

Brain. On opening the skull, the brain and meninges showed some injection of superficial vessels. In five cases schizonts were found in the endothelial cells of the small vessels. Experience showed that these endothelial parasites were best seen in Giemsa-stained impression smears made from a recently cut surface of the cerebrum. The cells lining the small vessels were grossly distended by the included schizonts, often sufficiently to block the lumen of the capillary. Certain smear preparations showed this very clearly. The dammed-up portions of the vessels were distended with blood cells, while below the obstruction the vessels were empty. Sections stained by the routine haematoxylin and eosin method showed the parasites, but excellent pictures were obtained by using Giemsa, especially if the sections were first mordanted with potassium permanganate and decolorized with oxalic acid.

Chronic cases

In the chronic, naturally infected, adult birds the carcasses were emaciated. The most marked changes found at post-mortem examination were in the spleen, kidney and liver. The cock from Songhor had parasites in the endothelium of the brain vessels. Furthermore this turkey had dry gangrene of the pendulous portions of the wattles: the hard, black, dead areas being still adherent to the live tissue, giving a picture not unlike that found in ergot poisoning.

The spleens were reduced in size, hard and fibrous. Microscopic examination showed gross collections of pigment and long-standing fibrosis. Only a few areas of follicular tissue remained.

The liver was firm and histological examination revealed chronic congestion with much fibrosis, the liver lobules having been attacked from the periphery inwards. This process was well shown in the small remaining areas of recognizable true liver tissue. Pigment was present.

The villi of the duodenal loop were packed with large granules of pigment. Large numbers of eosinophile cells were constantly present in the lungs, and to a less extent in the spleen and liver, of all the infected birds that were examined.

SIGNIFICANCE OF THE PIGMENT

The pigment found in the various organs failed to give a Prussian-blue reaction unless it was unmasked by sulphuric acid alcohol. Even after this treatment, only the smaller granules turned completely blue, whilst the larger collections remained greenish blue in the centres.

Considerable interest attaches to the constant presence of such large amounts of pigment in the organs. There was no pigment in young uninfected turkeys, but it was found in a few adult birds examined at this laboratory, although these had died from other causes. Through the favour of Mr N. Dobson of Weybridge, liver material was obtained from young turkeys that had died from other causes in England; no pigment could be found. Unfortunately, it has not been possible to obtain organs of adult turkeys from continental Europe for comparative examination. A large number of local domestic fowls have been examined without finding this pigment. In view of the occurrence of a similar pigment in human malaria it seems highly probable that the presence of the pigment in Kenya turkeys is a result of a plasmodial infection. It is so in young Kenya turkeys, as this has been checked by examining poults from a hatch before and after infection.

ATTEMPTS TO SET UP INFECTION IN OTHER AVIANS

Adult fowls proved refractory to artificial infection, but young 8-day-old chicks were susceptible.

Chicks 1, 2 and 3 were injected with infected turkey blood (0·2 c.c.) in the wing vein. Chick 1 was the only one to show parasites, and this was on the eleventh day. Blood from this chick was inoculated into chicks 3, 4 and 5. Turkey 304 was also inoculated with this blood and died of malaria 35 days later. Chicks 4 and 5 both showed parasites in their blood on the fourteenth day. Blood from chick 4 was subinoculated into three more chicks and into turkey 164. The turkey failed to react and was later proved to be susceptible, but two of these three chicks showed parasites in their blood. The blood of one of these (no. 6) was passaged into four other chicks. Only one of these, no. 12, showed parasites and its blood was put into turkey 98 which showed parasites the next day. This turkey survived 92 days and died of paralysis, and it seems to have been naturally infected before receiving the chick's blood. This well exemplified how difficult it was to be sure that even young turkeys obtained locally were 'clean'.

Both adult ducks and ducklings were inoculated with infected turkey and chick blood, but none showed parasites in the blood. Furthermore, the passaging of inoculated ducks' blood did not produce the disease in turkeys later proved to be susceptible.

None of the injected chicks showed any symptoms of illness following infection with this *Plasmodium*. The infection in the chicks' blood was *transient*, as shown by failure to infect a susceptible poult with the pooled blood of chicks 4, 5 and 8, 2 months after the parasites had been demonstrated in their blood.

DISCUSSION

It has only been possible to trace one reference to malaria in turkeys. This work, by Parcvanidze (1914), which has been unobtainable, is quoted by Giovannola (1939), and the parasite was designated *Plasmodium raupachi*. Macfie (1915) reported the finding of

Halterium sp. in a turkey which also had leucocytic inclusions. (The 'inclusion disease' was passaged through three generations in fowls, but the haemoprotozoa failed to infect fowls.) The turkey died. He states, 'a very few pigmented parasites were found in the red corpuscles' and presumably he was thus unable to trace the whole life cycle of this parasite in the blood. The two figures given are reminiscent of certain stages in the life-cycle of the parasite of turkey malaria. The relative disposition of the parasite and nucleus, the absence of any evidence that the microgamete was about to encircle the erythrocyte's nucleus, would suggest by the illustrations that he was dealing with a parasite similar to, if not identical with, that found in Kenya turkeys.

It seems unlikely that the *Plasmodium* referred to in this note can be a European parasite imported into Africa in turkeys, since were such an infection at all common in Europe it could hardly have been overlooked by investigators. What seems more probable is that the normal host of the parasite is some local wild-bird species or groups. Ross (1904–10) figured a *Plasmodium* of guinea-fowls (species not stated). His drawings, however, show that the nuclei of the parasitized red cells were displaced, which is not the case in the infection here described. Wenyon (1908) mentioned a *Plasmodium* of guinea-fowls (*Numida ptilorhyncha*), which he found in the Sudan, but he did not give a description of the parasite. The illustration showed a red blood cell containing a leucocytozoon and two *Plasmodia*. One was a young parasite and the other a schizont. Minchin (1910) found *Plasmodia* in *Numida ptilorhyncha*, and his coloured illustrations were of a parasite very similar to the turkey malaria *Plasmodium*. In Minchin's figures the nuclei of the erythrocytes are not displaced, and he also figures a cluster of free merozoites, which are often found in smears from turkey malaria. A further point to note is that all three of the above-quoted authors were examining material of Ethiopian origin; namely, Ross working in Kenya, Wenyon in the southern Sudan, and Minchin in Uganda. Kerandel (1909) stated he found *Haemoproteus* parasites in *Numida meleagris*, *Caprimulgus fossei* and *Strix flammea trimaculata*, but uses the term *Haemoproteus* (*Halterium*) in a general sense and gives no description of the parasites.

The probability that this turkey malaria is of local wild-bird origin is further strengthened by the fact that haemoprotozoa of birds usually have a wide range of vertebrate hosts. Thus, Ross (1898) successfully infected larks and crows with a *Plasmodium* obtained from sparrows, and Wenyon (1926) states *P. praecox* infects a wide range of birds including guinea-fowls.

Nothing is known of the natural method of spread of this parasite, and the few experiments made, using *culicine* and *anopheline* mosquitoes as intermediary hosts have, so far, given negative results.

Conclusions

1. A malarial disease of turkeys in Kenya is briefly described.
2. The disease induced by artificial inoculation of infective blood is highly fatal to young poults.
3. Schizonts are frequently to be found in 'exoerythrocytic' sites.
4. A prominent post-mortem feature of the infection is an accumulation of pigment in the spleen, liver, duodenal loop, and to a less extent in the lungs and kidneys of infected turkeys.
5. This parasite is harmless to ducks, adult fowls and chicks, although in the chick a symptomless, transient and apparently self-limited infection can be established.

I acknowledge the help received from Mr Dobson and Mr Poole, both of the Veterinary Laboratories, Weybridge; also the co-operation of Dr Carlton Herman of the New York Zoological Park, who has undertaken the study and recording of the life-cycle of this *Plasmodium*. I wish to thank my Director, Mr R. Daubney, for help and kind advice in carrying out this work.

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ON THE PERSISTENCE OF PROTOZOAN INFECTIONS IN THE CLOACA OF AN ANURAN, *XENOPUS LAEVIS* (DAUDIN)

By J. L. MOHR, *Department of Zoology, University of California, Berkeley*

Using the method developed by VanOrden & Nelson (1926) in withdrawing and examining cloacal contents of four captive individuals of the Plathander or clawed frog (*Xenopus laevis*), the writer determined that infections of ciliate protozoans had persisted for more than 6 years.

Prof. J. Frank Daniel* collected the four Plathanders near Capetown on 24 October 1935, taking them with him to Berkeley by way of Melbourne for experimental studies. They were maintained in the Vivarium of the Department of Zoology of the University of California separate from native Californian anurans. On 5 March 1940, the writer immersed each Plathander in one-eighth normal solution of magnesium chloride to relax anal musculature and then withdrew a portion of the cloacal contents of each with a pipette. Microscopic examination of the material withdrawn disclosed active individuals of four species of ciliates. Permanent preparations were made subsequently. All four species have reappeared as the *Xenopus* were examined several times each year up to the present.

There were numerous examples of the ciliate, *Paranyctotherus* (*Balantidium*) *kirbyi* Rodriguez (1939). Rodriguez described the spirotrich from smears made by Prof. Kirby from rectal contents of clawed frogs captured at Tradauw Pass, Cape Province, in 1934, and by de Villiers at Stellenbosch in 1938. Comparison with the type series† permitted positive identification of the species. Sandon (1941), working at Capetown with abundant materials, recently redescribed *P. kirbyi* establishing the genus *Paranyctotherus* to hold it.

A second, smaller ciliate, a typical *Balantidium*, probably the 'Balantidium entozoon' of Fantham (1919), was abundant both in the captive *Xenopus* and the Tradauw Pass smears. Prof. Sandon (personal communication) has seen this species along with *Paranyctotherus*. A *Protoopalina*, possibly *P. xenopodos* Metcalf (1923), was taken from two of the *Xenopus*; the same form is present in Rodriguez slides.

Because the Plathanders had no known avenue of infection by Californian ciliate parasites, because no protozoan found was characteristically Californian, and because three species of ciliates were identical with species present in preparations made by Kirby and de Villiers in Cape Province and a fourth falls within the bounds of Fantham's (1924) description of *Trichodina xenopodos* from the same host, it is concluded that this fauna is South African and that it has persisted for more than 6 years.

* The author is grateful to Prof. Daniel for opportunity to examine the *Xenopus*.

† The writer is indebted to Prof. Harold Kirby for use of several series of South African preparations.

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THE PARASITOLOGY OF HUMAN SCABIES

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(With 3 Figures in the Text)

Introduction

In human scabies the cuticle is invaded by all stages of the mite *Sarcoptes scabiei* de G. var. *hominis*.* Although there is a considerable literature on the disease there is little precise data on its parasitology. Diagnosis is frequently based not on the discovery of living mites but on general appearance of the patient; this need not lead to errors in diagnosis, but it often gives rise to a misunderstanding of the nature of parasitic infection. It is commonly believed that when a patient possesses numerous stigmata acari are present in immense numbers.

The data presented in this paper are the first approximately accurate figures which have been published on the parasite population of patients with scabies. They show the number of adult female *Sarcoptes* which occurred on 886 cases; all patients were soldiers.

An accurate knowledge of the numbers of adult female *Sarcoptes* on patients may be valuable in two ways: (1) in any statistical study of the epidemiology of scabies; (2) in the treatment aimed at killing all the parasites, which can be more easily achieved if we have more precise knowledge of their number.

Methods

In the 886 cases described here we believe that at least 95% of the adult female *Sarcoptes* have been located and extracted. The procedure is as follows: The patient lies naked on a couch in a good light in a warm room and the surface of the body is inspected, using a watchmaker's eyeglass; the mites are extracted with a mounted needle. With a little experience it is possible to detect the mites in the skin before removal. Each patient is carefully examined at least two, and often more, times, and the accuracy of the results has been ensured by keeping some patients from whom the mites have been removed in this way under observation for periods of weeks, to ensure that none has been missed.

This technique only estimates the adult female population and ignores the other stages (i.e. eggs, larvae, nymphs and adult males). Nevertheless, as the adult females probably bear a fairly definite relation to the whole mite population an estimation of this stage alone will give a good guide to the intensity of parasitic infection. We propose to refer to the number of adult females per patient as the 'parasite rate'.

The parasite rate

9978 mites were removed from 886 patients. Thus the average parasite rate was 11·3. Fig. 1 and Table 1 give some indication of the distribution of the different mite populations among the patients. 52% had under six parasites and only 3·9% had over fifty. Very occasionally large populations were encountered. For instance, one patient had 511 adult female mites.

* The most recent account of *Sarcoptes* has been given by Buxton (1941*a*).

In sarcoptic mange of animals many thousands of mites may occur on a single host. A similar state of affairs appears in man in 'Norwegian' or 'Crusted' scabies. We have not heard of a single case of anything approaching this condition occurring recently in Britain.

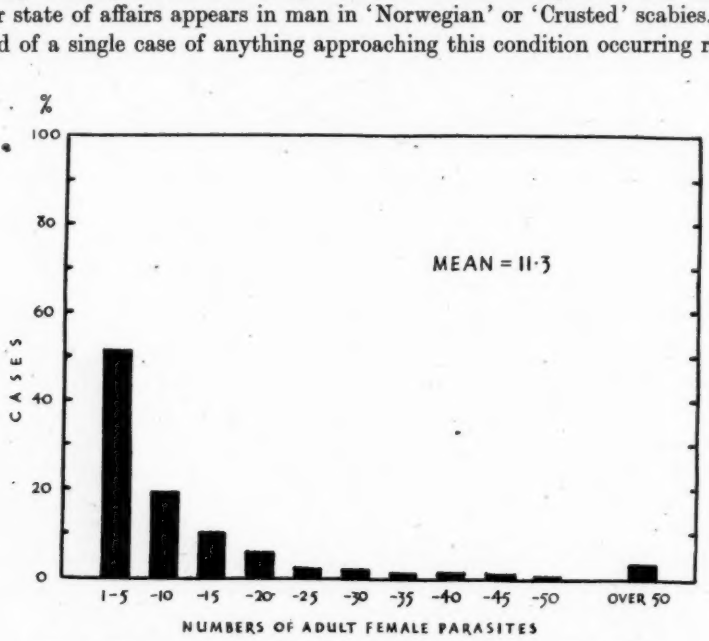

Fig. 1. The number of adult female *Sarcoptes* found on 886 cases of scabies.

Table 1. *Parasite rate in* 886 *cases of scabies*

Parasite rate (i.e. ♀ mites per patient)	No. of cases	%
1	137	15·5
2	123	13·9
3	74	8·4
4	68	7·7
5	54	6·1
1– 5	456	51·5
6– 10	170	19·2
11– 15	91	10·2
16– 20	48	5·4
21– 25	24	2·7
26– 30	21	2·4
31– 35	14	1·7
36– 40	13	1·5
41– 45	9	1·0
46– 50	7	0·7
51–100	28	3·2
Over 100 (i.e. 116, 166, 254, 280, 320, 511)	6	0·7

Sites of election

It is well known that certain sites on the body more frequently harbour mites than others, and this point, which has been specially investigated, is shown in Table 2 and Fig. 2. It will be seen that the majority, i.e. 63%, were found on the hands and wrists. The next most favoured site was the extensor aspect of the elbow, where 10·9% of the mites occurred.

These results may be expressed in a different way. If a patient is examined rapidly it may not be possible to search all the sites of election, and therefore it is valuable to know what proportion of cases can be diagnosed by examining certain areas only. The results expressed in Fig. 3 indicate what proportion of the total 886 cases had some mites

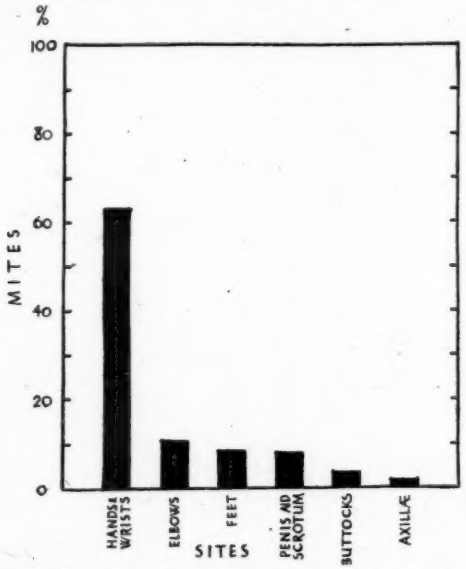

Fig. 2. Distribution of mites among the principal sites of election.

Table 2. *Sites of election: Number of adult female* Sarcoptes *extracted from the various sites. Total mites,* 9978. *Also frequency with which the different sites were affected. Total cases,* 886

Sites	Mites	%	Cases	%
Hands and wrists	6294	63·1	751	84·8
Elbows (extensor aspect)	1090	10·9	359	40·5
Feet and ankles	918	9·2	328	37·0
Penis and scrotum	842	8·4	319	36·0
Buttocks	398	4·0	140	15·8
Axillae	239	2·4	130	14·7
Knee	73	0·7	57	6·4
Navel	35	0·4	27	3·0
Hip	29	0·3	22	2·5
Thigh	22	0·2	19	2·2
Belly	18	0·2	13	1·5
Arm	13	0·1	7	0·8
Chest	10	0·1	10	1·1
Nipples	9	0·1	8	0·9
Back	3	—	2	0·2
Neck	3	—	2	0·2

on any particular site. Thus it will be seen that 85% had one or more parasites on the hands and wrists (mostly in addition to some parasites on other sites also). This means that at least nine out of ten of these cases of scabies could have been diagnosed by finding the causative organism if only the hands, wrists and elbows of the patient had been examined. This point may be of some importance when large groups of both sexes

have to be examined rapidly, though whenever possible the whole skin surface should be inspected.

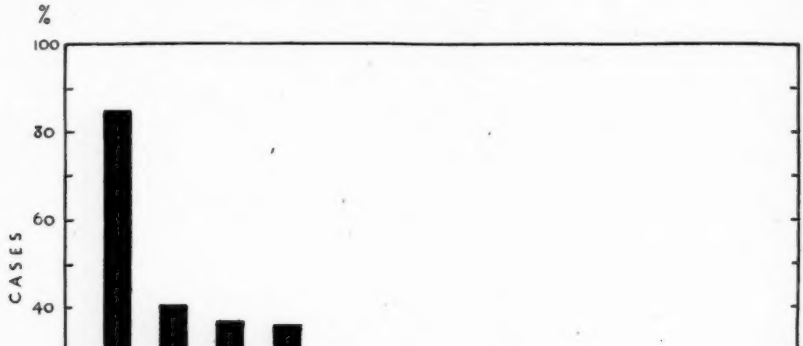

Fig. 3. The frequency with which various sites are infested with *Sarcoptes*.

Discussion

There appears to be little direct correlation between the general appearance of a scabies patient and the parasite rate. What is usually spoken of as a 'severe case', covered with follicular pustules and boils, will often have far fewer parasites than a patient with practically no clinical symptoms. Few of our cases had sufficient secondary infection of the skin to necessitate prolonged hospital treatment; it seems that at the present, in the army, scabies is being diagnosed before serious secondary complications occur (far worse complications are being regularly discovered in civilians, particularly children, among hospital out-patients). Of the 886 cases considered here, only forty had at all widespread secondary infection, and among these men the parasite rate was 12. Here the parasites were usually discovered on those parts of the body which were *not* secondarily infected, for the formation of pus appears to kill mites with which it comes in contact. Incidentally, the worst secondary infection was observed in men who had had scabies but had since been overtreated at other centres with sulphur (usually by the 'thiosulphate-HCl' method).

Of the 886 patients, 585 (66%) had detected symptoms which had caused the men to report sick, whereas 301 (34%) had been diagnosed at routine medical inspections. There was no significant difference in the parasite rate in these two groups. Some of the patients stated that even when sent for treatment they had no unpleasant itchy symptoms, whereas others reported that itching had been felt for periods of up to 12 weeks. There was no significant difference in the parasite rate of patients who had noticed itching for 1, 2, 3, 4 weeks, or for longer periods.

Those familiar with clinical scabies may be surprised at the results given in Table 2 and Fig. 2, which state that 63% of the mites occur on the hands, whereas only 8% are found on the genitals and a mere 4% on the buttocks. They will be inclined to state that far more obvious stigmata are found on the genitals and buttocks than on the hands. This is true also of the patients considered here, but careful observation showed that on many softer parts of the body, although very obvious old burrows could be found, these were seldom occupied by mites. It appears that in the genitals, buttocks and axillary region the skin gives a severe reaction to the parasites, and this reaction causes the patient to scratch and so the mite may be removed. Even after its removal the site of the old burrow may remain irritant for a considerable period; thus though only 37% had live mites on the genitals nearly 100% showed the remains of old burrows in this region. On the hands, however, the reaction is often comparatively slight even when the mites are numerous. If the hands are kept clean, little secondary infection may occur, and as ordinary washing has little effect on the infestation (Mellanby, 1942*a*) a fairly large population of mites may remain there.

The results given here refer to the parasites found on cases of scabies at the time of treatment. Results describing the development of the population of *Sarcoptes* and the symptoms of scabies will be published shortly, and at the moment it need merely be stated that the numbers of parasites by no means always show a steady increase as the disease proceeds, for rapid falls in population are very frequent. From the point of view of the epidemiology, the patients with large numbers of parasites will probably have the greatest importance, and the 3·6% with over fifty parasites may well be a greater danger to the community than the 52% with light infestations of under six adult females.

This investigation has been made on adult male patients only. A smaller number of females and children with scabies has been examined, and no striking differences in parasitology have been observed.

It is interesting to compare the populations of *Sarcoptes* on man with that of other human parasites. Both head and body lice (see Buxton, 1941*b*; MacLeod & Craufurd-Benson, 1941; Mellanby, 1942*b*) usually occur in small numbers, not unlike those described for *Sarcoptes* in this paper, with occasionally much larger populations on particular individuals.

SUMMARY

1. An analysis has been made of the numbers and location of adult female *Sarcoptes scabiei* found on 886 men suffering from scabies.

2. The average number of mites per man was 11·3. 52% of the cases had under six mites and only 3·6% over fifty.

3. 63% of the mites were found on the hands and wrists, 10·9% on the elbows, 9·2% on the feet, 8·4% on the genitals and 4% on the buttocks.

4. So-called 'severe' cases of scabies, showing widespread clinical symptoms, may have very few parasites, whereas cases with few symptoms may have large mite populations.

To obtain the results given above necessitated many hours of tedious work. The greater part of this was done by Mr W. C. Bartley, Corporal G. A. Burns, R.A.M.C., and by volunteers of the Sorby Research Institute. Part of the expenses were paid by grants from the Ministry of Health and the Medical Research Council.

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FURTHER WORK ON THE COMPARATIVE EFFICACY OF DIFFERENT CULICIFUGES UNDER LABORATORY CONDITIONS

By D. N. ROY and S. M. GHOSH, *School of Tropical Medicine, Calcutta*

A preliminary report on the results of our studies on the comparative efficacy of different culicifuges under laboratory conditions has been published (Roy, Ghosh & Chopra, 1942).

We now present in tabular form additional information on the culicifuge action of other substances which are often recommended for the purpose.

Tests have also been performed with hungry *Aëdes aegypti*, whose propensity to bite man is very marked, to determine the extent of deterioration which some of these preparations are liable to undergo at the end of a fortnight, when stored in ordinary glass phials and left on the laboratory bench.

Table

Composition and author	Quantity used	Period of effectiveness in min.		Extent of deterioration in 2 weeks %
		Aëdes aegypti	*Armigeris obturbans*	
Dover, C.: Citronella oil, ½ oz. Spirits of camphor, ½ oz. Cedarwood oil, ½ oz. White petroleum jelly, 2 oz.	Small Large	Nil 3	10 45	100
Bamber oil: Citronella oil, 1½ pt. Liquid paraffin or kerosene, 1 pt. Coconut oil, 2 pt. Carbolic acid, 1%	Small Large	10 30	40 75	0
Covell, G.: Vaseline, 100 pt. Naphthaline, 10 pt. Camphor, 1 pt.	Small Large	Nil 20	4 50	50
Macnay, C. G.: Concentrated extract of pyrethrum, ½ oz. Castor oil, 4 oz. Citronella oil, 5 drops	Small Large	210 More than 300	270 More than 300	50
Boyd, J. E. M.: Menthol, 1 dr. Turpentine oil, 1 oz.	Small Large	Nil 25	5 40	66
Boyd, J. E. M.: Citronella oil, 1 oz. Spirits of camphor, 1 oz. Cedarwood oil, ½ oz.	Small Large	10 35	25 50	80
Patterson, T. C.: Citronella oil, 18·25% Camphor, 1% Cedarwood oil, 9% Paraffin durum, 26·75% Paraffin molle alba, 45%	Small Large	5 40	15 60	80
Patterson, T. C.: Turpentine oil	Small Large	Nil 30	30 40	0
Patterson, T. C.: Juice of fresh lime. The treated part was allowed to dry	Adequate	Nil	Nil	—

Table (*cont.*)

Composition and author	Quantity used	Period of effectiveness in min.		Extent of deterioration in 2 weeks %
		Aëdes aegypti	*Armigeris obturbans*	
Patterson, T. C.: Pine oil	Small	5	15	0
	Large	25	40	
Patterson, T. C.: Cod-liver oil	Small	5	10	0
	Large	12	35	
For Army in India: Camphor, 1%, Citronella oil, 27%, Hard paraffin, 25%, Soft paraffin, 42%, Extract of pyrethrum liquid, 5%	Small	Nil	20	33
	Large	20	45	
Syddiq, M. M.: Beeswax, 2 dr., Spermaceti, 4 dr., Coconut oil, 4 dr., Fuller's earth, 4 dr., Liquid paraffin, 1 oz., Citronella oil, 1 oz., Carbolic acid, 10 drops	Small	Nil	10	50
	Large	20	35	
Howard, Dyar & Knab: Sulphate of potash, 5% solution	Small	3	3	0
	Large	7	10	
Cream prepared with pyrethrum extract and Cassia oil. Reaction: strongly alkaline	Small	Nil	Nil	100
	Large	10	15	
Cream prepared with pyrethrum extract and pine oil. Reaction: faintly alkaline	Small	Nil	Nil	50
	Large	15	25	
Cream prepared with clove oil, Cassia oil, ground-nut oil and extract of pyrethrum. Reaction: neutral	Small	Nil	Nil	35
	Large	25	45	
Cream containing pyrethrum showing marked acid reaction	—	Nil	Nil	—
Camphor, 5 gr., Vaseline, 1 dr.	Small	Nil	Nil	100
	Large	10	15	
Cashewnut-shell oil	Small	Nil	Nil	—
	Large	Too sticky		
Barium carbonate, 10 gr., Vaseline, 1 dr.	Small	Nil	Nil	0
	Large	5	10	
Castor oil	Small	15	18	0
	Large	Too sticky		
Juice of leaves of cherry gold	Small	Nil	20	—
	Large	15	90	
Eucalyptus oil	Small	45	70	0
	Large	90	150	
Orange-peel juice	—	Nil	Nil	—
Soap (ordinary bar soap, after application the parts were allowed to dry)	Thin	Nil	Nil	—
	Thick	Nil	Nil	
Linseed oil	Small	Nil	Nil	0
	Large	10	25	
Juice of leaves of marigold	Small	5	40	—
	Large	60	90	
Liquid paraffin	Small	5	5	0
	Large	10	30	
Neem oil	Small	5	30	0
	Large	20	65	
Neem oil, Extract of pyrethrum liquid 1 : 20, Citronella oil—in equal parts	Small	90	100	30
	Large	150	240	
Tobacco leaf extract prepared by boiling dried leaves. The parts were allowed to dry after application	—	Nil	Nil	—

Table (*cont.*)

Composition	Quantity used	Period of effectiveness in min.		Extent of deterioration in 2 weeks %
		Aëdes aegypti	*Armigeris obturbans*	
Ammonium carbonate—rubbed on the skin	—	Nil	Nil	—
Pine tar, 2 gr. Olive oil, ½ dr.	Small Large	20 50	45 70	0
'Shell Tox' (B.O.C.)	Small Large	10 90	30 105	45
'Flit'	Small Large	15 70	15 105	45
Cedarwood oil	Small Large	Nil 15	Nil 35	0
Clove oil	Small Large	90 210	150 240	0
White vaseline	Small Large	Nil Nil	Nil Nil	—
Deobase oil	Small Large	Nil Nil	Nil Nil	—
Oil coriander	Small Large	50 90	70 120	0
Oil caraway	Small Large	40 65	45 95	0
Oil anisi	Small Large	20 35	27 45	0
Oil ajwan	Small Large	20 45	30 60	0
Oil geranium	Small Large	35 70	50 90	0
Oil myriasticae	Small Large	10 25	17 35	0
Oil juniper	Small Large	15 25	17 30	0
Oil cajuputae	Small Large	Nil 10	15 25	0
Oil cade	Small Large	Nil 10	Nil 15	0
Oil neroli	Small Large	11 25	15 32	0
Oil santali	Small Large	Nil 20	12 35	0
Oil chamomile	Small Large	Nil 10	8 20	0

Conclusion

Any preparation containing pyrethrum will no doubt afford considerable protection against mosquito bites but, as pyrethrum undergoes marked deterioration when exposed to light, its use must be limited. It is on this account that the preparation used in the army in India is not stable. Apart from this disadvantage, it contains wax and thus it is unsuitable for application during the humid weather in the tropics when malaria is widely prevalent.

Bamber oil is quite stable but its action is short lived.

We have not had ample opportunities of testing pyrethrum when prepared in the form of a cream. Limited observations indicate that pyrethrum deteriorates rapidly in acid medium, but when the reaction is neutral its action lasts comparatively longer.

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NEW PARASITIC MITES (ACARINA)

By CHARLES D. RADFORD*

(With 11 Figures in the Text)

Introduction

The genus *Eulaelaps* was raised by Antonio Berlese in 1903 to include the species of Laelaptidae in which the genito-ventral scutum of the female was large and extended to the anal scutum, and which possessed a large triangular meta-podal scutum posterior to coxa iv.

On checking through the literature on the known species of this genus to verify the validity of a species which I thought to be new (herein described as *Eulaelaps citellus* n.sp.), I found one which must be transferred to a new genus.

This is necessary, as the species described by Pearse (1930) does not conform to the above description of the genus: (*a*) it does not possess the meta-podal scuta; (*b*) the sternal, genital and ventral scutae are fused into a single scutum covering almost the entire ventral surface; (*c*) the posterior margin of this ventral scutum is impressed so as almost to surround the anal scutum.

Pearse also states 'Legs, setose distally, 8 segmented', which seems unusual in the Laelaptidae, unless he mistakes the pseudo-division of the femur and tarsus. In these segments of the legs there is a faint line round the joint which might be mistaken for a dividing line, but these lines are not true articulated joints. It is thought, therefore, that the statement is not quite correct, the legs possessing only six articulated segments.

This species, *Eulaelaps ewingi* Pearse, 1930, has, therefore, been made a monotypic species and placed in a new genus, *Ligialaelaps*.

Nathan Banks (1909), in his description of *Laelaps propheticus*, refers to its similarity to *L. stabulans*. The latter is *Eulaelaps stabularis* Koch, 1836, used by Berlese (1903) as the generic type. Also the figure given by Banks shows the meta-podal scutum posterior to coxa iv, which establishes its place in the genus *Eulaelaps*. In the same paper Banks (1909) describes and figures a second specimen under the name *Laelaps pedalis* which, for similar reasons, must be brought into the genus *Eulaelaps*.

A third specimen (*Laelaps longiseta*) described in this paper (Banks, 1909) is not proper to the genus *Laelaps* sensu stricto because: (i) the ventral scutum possesses more than four pairs of spines; (ii) the anal scutum possesses more than three spines. This specimen is therefore made the type species of the new genus *Banksia*. Hirst (1923*a*) published a short paper describing *Laelaps parvulus*, *vansomereni* and *bakeri*, but only the females were described and no figures accompanied the descriptions.

Amongst material sent to me by the Zoological Survey Committee, Onderstepoort, South Africa, I have found females of *Laelaps parvulus* and what is undoubtedly the male, and have herein described and figured the sexes.

Hirst (1915) published a description, together with figures, of a mite found in the London Zoo on a Couper's snake. The females only were found and described as *Ichoronyssus serpentium* n.sp. Ewing (1922) raised a new genus *Serpenticola* to accommodate

* Work carried out with the aid of a Government Grant.

this species, overlooking the genus *Ophionyssus* Megnin, 1884. As Hirst's paper described only the female, I take this opportunity of issuing the description and figure of the male. Both sexes were taken on a pet rainbow boa (*Epicrates cenchris* L.) in Manchester.

The species herein described as new are: *Eulaelaps citellus* n.sp., from a squirrel (*Citellus beecheyi*); *Haemolaelaps mystromys* n.sp., from a rat (*Mystromys albicaudatus*); *Listrophoroides dasymys* n.sp., from a rat (*Dasymys* sp.); *Liponyssus dendropicos* n.sp., from a woodpecker (*Dendropicos fuscescens lapidus*).

Genus **Eulaelaps** Berlese, 1903

Eulaelaps citellus n.sp.

The female venter (Fig. 1) shows the specimen to be broadly oval with distinct shoulders level with the anterior margin of sternal scutum. Sternal scutum shield-shaped, slightly longer than broad, extending from anterior of coxa ii to middle of coxa iii. Anterior margin concave medially with a concavity between first sternal spine and antero-lateral corner. Lateral edge of scutum deeply concave round coxa ii, postero-lateral corner projecting between coxae ii and iii. Posterior margin of scutum extending backwards to middle of coxa iii, concaved medially. Close to the anterior margin of this scutum is a pair of pre-sternal scuta or jugularia.

First pair of sternal spines close to the anterior margin of sternal scutum in the convex portions. Second pair placed about two-thirds of the distance from anterior to posterior margin, with the third pair close to the posterior margin. The scutum also possesses the usual two pairs of pores, the first close to the first pair of sternal spines, the second between the second and third pair of spines.

In this species the genital and ventral scuta are fused in the middle, posterior to coxa iv. Anterior edge of genital scutum indistinct. The genital pair of spines is placed level with posterior edge of coxa iv. Ventral scutum extends from genital scutum to anterior of anal scutum and occupies most of the area posterior to coxa iv. The scutum bears about fifty-nine spines which are shorter and more slender than the sternal and genital spines. In the specimen figured some of the spines are missing but the spine bases can be seen.

Anal scutum broadly triangular, its anterior edge with a medial convexity which projects into the posterior border of the ventral scutum. Anal pore placed in the centre of the scutum, paired anal spines level with middle of anal pore, unpaired anal spine near to apex of scutum.

Spines on soft integument flanking ventral and anal scuta, thirty-four to thirty-eight. Meta-sternal spines not borne on meta-sternal scuta but placed close to third pair of sternal spines, with third pair of pores flanking them. Meta-sternal scutum extending from postero-lateral corner of sternal scutum to middle of coxa iv with a long projection between coxae iii and iv.

Meta-podal scutum triangular and placed posterior to coxa iv. All ventral scuta are reticulated. Stigmal pore level with coxa iv with peritreme reaching forward to anterior of coxa i.

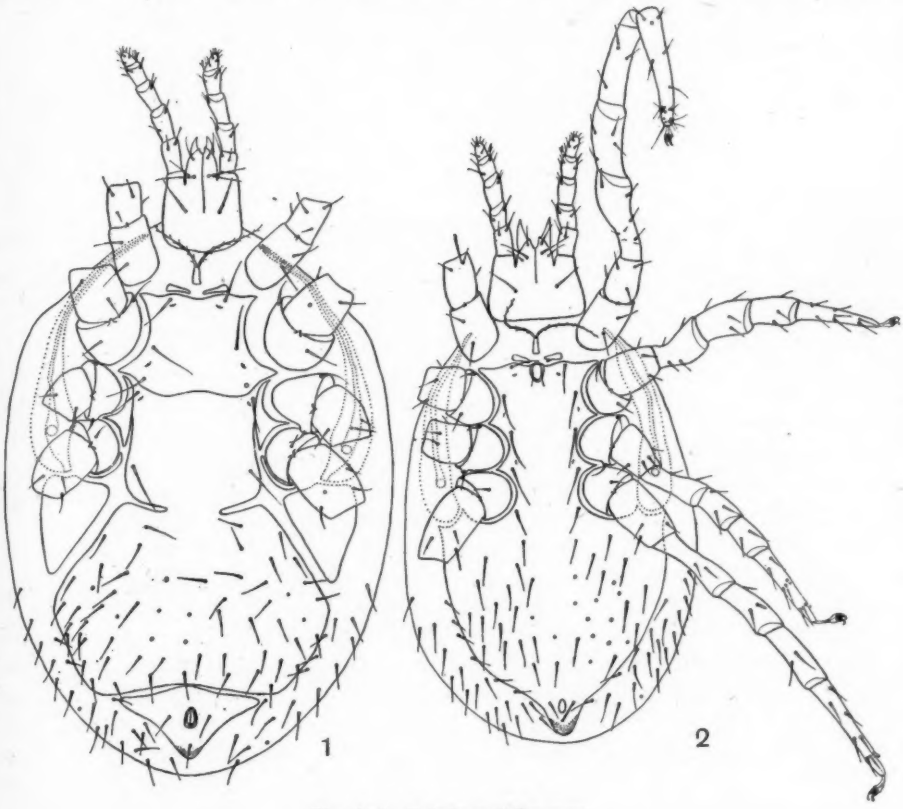
Legs i and iv longer than ii and iii, leg iii the shortest, leg ii stouter than the remainder. Chaetotaxy of legs normal.

Dorsum completely covered by dorsal scutum. Dorsal spines numerous but not as long as the spines of the venter.

The male venter (Fig. 2) shows the specimen to be oval, with shoulders less distinct than

in the female. Ventral scutum entire, reticulated, occupying most of the area between coxae ii, iii and iv and extending almost to the posterior end of the body, leaving an exposed area laterally. Anterior to the ventral scutum is a pair of pre-sternal scuta flanking the genital pore which lies in the centre of the anterior border of the ventral scutum.

The first pair of sternal spines is placed near the anterior margin in the convexity. Second pair of sternal spines level with middle of coxa ii, third pair close to anterior edge



Figs. 1, 2. *Eulaelaps citellus* n.sp.
Fig. 1. Female venter. Fig. 2. Male venter.

of coxa iii. First pair of pores lying between, and posterior to, first pair of sternal spines. Second pair of pores between second and third pair of sternal spines. Meta-sternal pair of spines between coxae iii and iv with third pair of pores anterior to them, closer together. Genital pair of spines level with posterior of coxa iv.

Spines of ventral portion of scutum, posterior to coxa iv, about forty in number, not as long as first five pairs of ventral spines. Anal pore placed some distance in front of posterior tip of scutum, paired anal spines level with anterior end of pore, with unpaired spine posterior to anal pore.

Flanking the ventral scutum, posterior to coxa iv, there are some twenty to twenty-

three pairs of spines on the soft integument of venter. Stigmal pore level with anterior of coxa iv, peritreme reaching forward to middle of coxa i.

Dorsum entirely covered by dorsal scutum, bearing numerous spines, not quite as long as the ventral spines. Legs i and iv the longest, leg i stouter than the others, leg iii the shortest. Chaetotaxy of legs normal.

Host. A squirrel (*Citellus beecheyi* Richardson).

Locality. Contra Costa, California, U.S.A. March 1936.

Measurements. ♀ 1·15 × 0·82 mm.; ♂ 0·95 × 0·61 mm. (without capitulum).

Eulaelaps propheticus (Banks 1909) emend Radford.

This species, described by Banks (1909) as *Laelaps propheticus*, should be taken out of the genus *Laelaps* as it appears, both from the figure and description given by Banks, to belong to the genus *Eulaelaps*. From the shape of the ventral scutum and the presence of a distinct anal shield it would appear to be a female, though in the original description it is referred to as a male.

The number of spines on the ventral scutum and the presence of a distinct meta-podal scutum warrants its inclusion in the genus *Eulaelaps*; also Banks in his description says 'Similar to *L. stabulans*', by which he means *Eulaelaps stabularis* Koch, 1836.

Host. A groundhog.

Locality. Guelph, Ontario, Canada.

Measurement. 1·0 mm.

Eulaelaps pedalis (Banks, 1909) emend Radford.

The figure given by Banks (1909) shows the presence of a distinct meta-podal scutum, and the ventral scutum possesses about twenty-one pairs of spines, which warrants its being placed in the genus *Eulaelaps*, and not *Laelaps*.

Host. A chipmunk.

Locality. Guelph, Ontario, Canada.

Measurement. 1·0 mm.

Pearse (1930) described and figured a species of mite taken in large numbers (about eighty specimens, Cat. Nos. 62837, 62838 U.S. Nat. Mus.) from the abdominal appendages of a Japanese crustacean (*Ligia exotica* Roux) to which he gave the name *Eulaelaps ewingi*. From his paper it would appear that the specimens warrant a new genus as they in no way fit the genus *Eulaelaps* Berlese 1903, for the following reasons: (i) the absence of the meta-podal scutum posterior to coxa iv; (ii) the absence of distinct and separate sternal, genito-ventral and anal scuta; (iii) the venter being covered by a fused sterno-genito-ventral scutum which bears a concavity on its posterior end to accommodate the anal scutum. No mention is made of the male, the figures being of the female. As the specimen does not resemble any known genus of the Laelaptidae it is proposed to raise a monotypic genus *Ligialaelaps*.

Genus **Ligialaelaps** n.g.

Body flattened, dorsum covered by dorsal scutum, eighteen pairs of dorsal setae. Sternal and genito-ventral scuta united, extending from anterior edge of coxa ii to coxa iv, then widening, covering most of area posterior to coxa iv, reaching end of venter and surrounding antero-lateral edge of anal scutum. Ventral scutum furnished with about six pairs of setae. Anal scutum wider than long, semi-circular, bearing three anal setae.

Chelicera with two teeth on both the fixed and movable finger. Second segment of palp bears two stout, thorn-like spines on dorsal surface.

Type species. *Eulaelaps ewingi* Pearse, 1930.

Ligialaelaps ewingi (Pearse, 1930) emend Radford.

The most conspicuous feature of this species is the fused sternal and genito-ventral scuta, extending from anterior of coxa ii to coxa iv where the scutum widens to occupy most of the area posterior to coxa iv, extending to posterior tip of venter and surrounding the anal scutum. The three pairs of sternal setae in Pearse's figure appear to be placed further back than is usual in the Laelaptidae. The first pair are level with coxa ii instead of being on or near the anterior margin of the scutum. This is the position usually occupied by the second pair of sternal setae. The third pair are in the position appropriate to the meta-sternal setae. Fourth pair of setae, presumably the genital setae, are level with coxa iv. Two further pairs of setae are shown posterior to coxa iv. It would seem reasonable to assume that the first pair of sternal setae are missing, which would then bring the four pairs in their relative positions. Anal scutum broader than long, semi-circular and fitted into the ventral scutum. The usual three anal setae are present. Stigmal pore between coxae iii and iv, peritreme reaching forward to coxa i. Chelicera furnished with two teeth on both the fixed and movable finger. Long hyaline process on movable finger of chelicera as in the genus *Haemolaelaps*. Second segment of palp bears two stout, thorn-like spurs on the dorsal surface. Dorsum covered by dorsal scutum and furnished with about eighteen pairs of setae.

Host. A crustacean (*Ligia exotica* Roux).

Locality. Aburatsubo, Japan. 16 August 1929.

Measurement. ♀ 0·47 × 0·35 mm.

The species described by Banks (1909) as *Laelaps longiseta* would appear to need to be put in a new genus, as his figure shows the ventral scutum to possess some twenty-one pairs of spines, the anal scutum possesses some eleven (or more) pairs of spines. The absence of meta-podal scuta posterior to coxa iv precludes it from the genus *Eulaelaps*.

It is proposed therefore to raise a new genus *Banksia*.

Type species. *Laelaps longiseta* Banks, 1909.

Genus **Banksia** n.g.

Body longer than broad, spines on dorsal scutum numerous, long. Dorsal scutum possessing many fine, short spines posteriorly.

On shoulders there is a pair of very long spines. A similar long spine is borne dorsally near the distal end of femur iv. Ventral scutum wide, almost filling the area posterior to coxa iv, possessing about twenty-one pairs of spines. Anal scutum wider than in other Laelaptidae, almost covering posterior end of venter, and bearing about eleven pairs of spines.

Type species.. *Laelaps longiseta* Banks, 1909.

Banksia longiseta (Banks, 1909) emend Radford.

Body longer than broad, dorsal scutum covering dorsum and possessing numerous long spines, with a number of smaller, finer spines round posterior edge of scutum. Laterally,

at level of shoulders, is a pair of very long, stout spines. Dorsally, at distal end of femur iv, there is a similar spine.

Ventral scutum almost completely covers the area posterior to coxa iv, its posterior edge widely concave. Scutum possessing about twenty-one pairs of spines. Anal scutum broader than long, anterior edge convex, conforming to the shape of the posterior edge of ventral scutum, from which it is narrowly separated. About eleven pairs of spines on anal scutum.

From Banks's figure it is not clear whether the usual three anal spines are present. A narrow strip of soft integument flanks the ventral and anal scuta and bears a number of spines.

Host. A carrion beetle.

Locality. Guelph, Ontario, Canada.

Measurement. 0·9 mm.

Genus **Haemolaelaps** Berlese, 1910

Haemolaelaps mystromys n.sp.

The female venter (Fig. 3) shows the sternal scutum to be wider than long, anterior margin almost straight between first pair of sternal spines, then diverging outwards and forwards, with antero-lateral corners level with anterior of coxa ii. Sternal scutum concave laterally, postero-lateral corners level with posterior of coxa ii. Posterior edge of scutum extending backwards to middle of coxa iii, with a wide concavity medially.

First pair of sternal spines close to the anterior margin of scutum, second pair midway to posterior margin of scutum, third pair close to posterior margin of scutum. The sternal scutum also possesses two pairs of pores.

Meta-sternal spines level with posterior edge of coxa iii, flanked laterally by the meta-sternal scuta. Third pair of pores level with middle of coxa iii and anterior to meta-sternal spines.

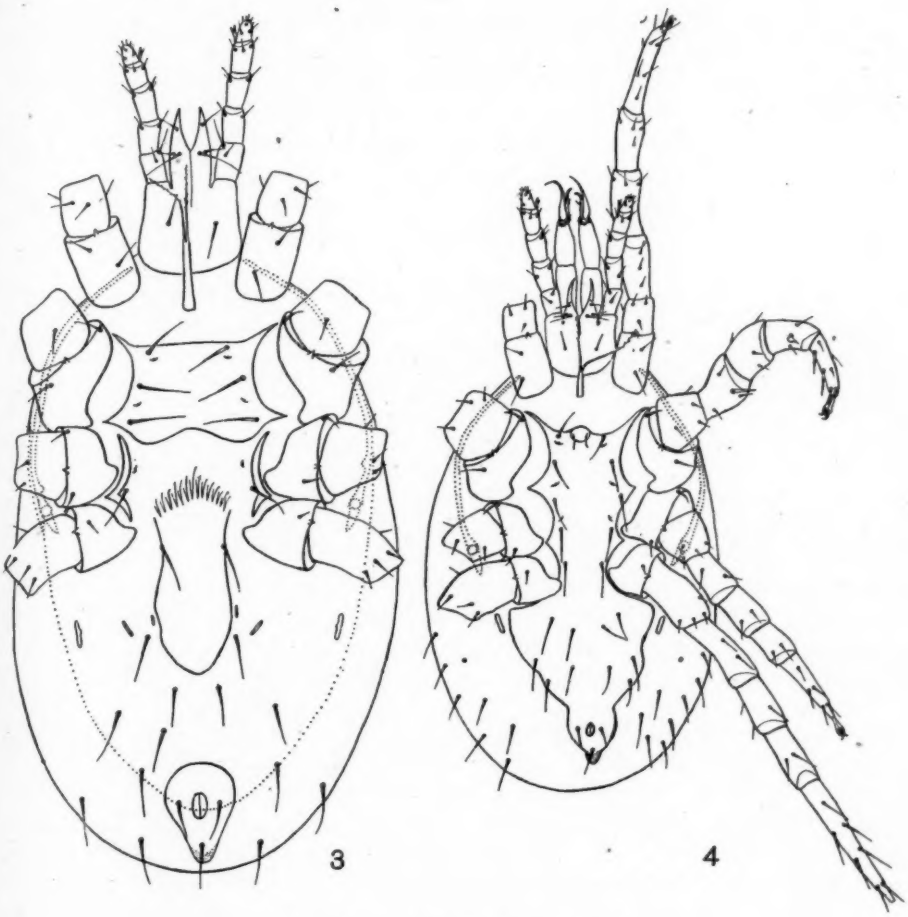
Genito-ventral scutum long, narrow, extending half-way to the posterior edge of venter, its anterior margin striated, the genital pair of spines level with coxa iv. Flanking the genito-ventral scutum there are two pairs of small scuta, with a much larger scutum closer to the lateral border of the body.

Anal scutum pear-shaped, large, with the anal pore placed in centre of scutum. Paired anal spines level with centre of pore unpaired anal spine placed well back towards posterior tip of scutum. Uncovered area of venter bearing seven pairs of spines, the first and second pairs flanking the genito-ventral scutum. Stigmal pore between coxae iii and iv, peritreme reaching forwards to anterior of coxa i. Legs i and iv longer than ii and iii, legs ii stoutest, legs iii shorter than the others. Dorsal scutum (shown in dotted line) not covering entire dorsum, dorsal spines few.

Male venter (Fig. 4) shows anterior margin of ventral scutum deeply concaved, genital pore placed in the centre, with antero-lateral corners projecting laterally to anterior edge of coxa ii. Scutum deeply concaved round coxae ii, iii and iv, with lateral projections between coxae ii and iii, and between iii and iv. Scutum expanded posterior to coxa iv where it reaches its maximum width, then gradually reducing to anterior of anal pore.

First pair of sternal spines close to anterior edge of ventral scutum. Second pair of spines level with middle of coxa ii, third pair level with posterior edge of coxa ii. Meta-sternal pair of spines slightly posterior to middle of coxa iii. Genital pair of spines placed

well forwards level with coxa iv. Posterior to coxa iv there are five pairs of spines not including the three anal spines. Anal pore in centre of pear-shaped end of scutum, paired anal spines level with middle of pore. Unpaired anal spine midway between pore and posterior tip of scutum.



Figs. 3, 4. *Haemolaelaps mystromys* n.sp.
Fig. 3. Female venter. Fig. 4. Male venter.

The first pair of pores is placed close to the first pair of sternal spines, second pair of pores between second and third pair of sternal spines, with the third pair of pores anterior to meta-sternal pair of spines. Flanking the ventral scutum at its maximum width is a pair of elliptical platelets. Soft integument of venter bearing some eight or nine pairs of spines. Stigmal pore level with coxa iii, the peritreme extending forwards to middle of coxa i.

Chaetotaxy of legs normal except legs ii. On venter of femur ii there is a stout spine

distally, whilst on the tibia there is a peculiarly shaped spur, and the tarsus bears three or four stout spines ventrally. Dorsum not completely covered by dorsal scutum, spines few, the posteriormost pair of dorsal spines long.

Host. A white-tailed rat (*Mystromys albicaudatus* Smith).

Locality. Glen Craig, Albany, South Africa. 31 October 1939.

Measurements. ♀ 0·97 × 0·61 mm., ♂ 0·7 × 0·35 mm. (without capitulum).

Genus **Laelaps** Koch, 1842

Laelaps parvulus Hirst, 1923.

Venter of female (Fig. 5) shows the sternal scutum to be almost square, anterior margin of scutum almost straight between first pair of sternal spines, with a concavity on each side, antero-lateral corners projecting outwards and forwards. Lateral edge of scutum concave, postero-lateral corner rounded, posterior margin of scutum level with middle of coxa iii, and with three concavities.

First pair of sternal spines on anterior margin of scutum, the second pair placed on middle line of scutum, third pair close to postero-lateral corner. Meta-sternal pair of spines borne on meta-sternal scuta between coxae iii and iv. These four pairs of spines are exceptionally long and slender. The three pairs of pores are placed in the usual positions.

Genito-ventral scutum widened posterior to coxa iv, posterior margin of scutum almost straight, and bearing the usual four pair of spines. Anal scutum large, pear-shaped, with anterior edge almost straight, anal pore placed in anterior third of scutum. Paired anal spines level with posterior of pore, unpaired anal spine placed well back towards the apex of scutum. Anal spines stout. Some fifty-one to fifty-five pairs of spines are borne on the soft integument of the venter, of which there is a line of about six close to coxa iv which are shorter than the others. There is a pair of short, stout spines on the ventral surface of the capitulum. Legs short and stout, legs i shorter than the others. Coxa i bears two stout spurs on the ventral surface, with a stout spur on the proximal posterior corner of the trochanter. Anterior edge of coxa ii bears a stout, tooth-like spur, with a stout spur on the ventral surface close to this tooth-like spur, and a stout spur on the posterior edge. Trochanter ii also bears a stout spur close to the posterior spur of the coxa. Tarsus ii furnished with three stout spurs near its distal end.

Two stout spurs ventrally on coxa iii, one anterior the other posterior. Distal end of tibia bears a stout spine, tarsus bearing six stout spines. Coxa iv bears only a single stout spur in the middle, a stout spur is borne on the trochanter at the posterior distal corner. Spur on proximal anterior edge of tibia longer than the others on this segment. Anterior edge of tarsus iv bears three long, stout spines.

There is a comb-like process on the anterior third of coxae ii and iii ventrally, before the first spur. On coxa iv the comb-like process bears a long tooth-like projection in place of the stout spur present on coxae ii and iii.

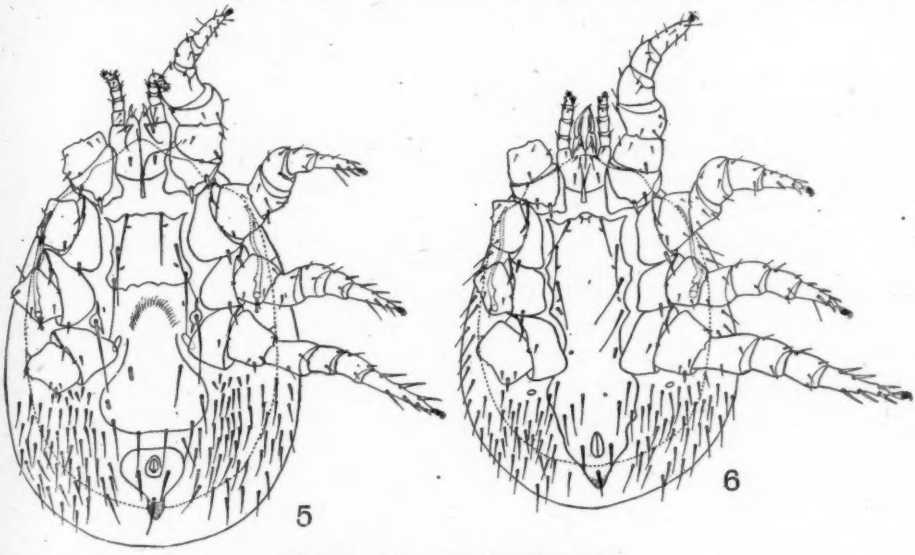
Stigmal pore level with coxa iii, peritreme extending to middle of coxa ii.

Dorsal scutum (shown in dotted line) distinctly shouldered at level of coxa i, does not completely cover the dorsum. Spines of dorsal scutum few, shorter and finer than ventral spines, except for those spines round the posterior edge which are somewhat longer.

The dorsal surface of femur i bears four long, stout spines two of which are exceptionally long.

The male venter (Fig. 6) shows the anterior margin of the ventral scutum to be concave on each side of the genital pore. Scutum concave laterally around coxa ii, irregularly concave around coxa iii, with a lateral projection between coxae iii and iv. The scutum is then constricted to the level of the genital pair of spines, whence it commences to widen, reaching its maximum width posterior to coxa iv.

First pair of sternal spines flanking genital pore and close to anterior edge of scutum, second pair of sternal spines level with the middle of coxa ii. Third pair of sternal spines are anterior to the middle of coxa iii. Meta-sternal pair of spines between coxae iii and iv, with genital pair of spines posterior to the middle of coxa iv. As in the female, these four pairs of spines are longer than the remainder of spines on ventral scutum. On the



Figs. 5, 6. *Laelaps parvulus* Hirst, 1923.
Fig. 5. Female venter. Fig. 6. Male venter.

ventral portion of the scutum, posterior to coxa iv, there are nine spines in addition to the three anal spines.

Anal pore large, situated its own length from posterior tip of scutum, paired anal spines level with middle of pore, unpaired anal spine midway between pore and apex of scutum. In addition the ventral scutum possesses the usual three pairs of pores. Flanking the ventral scutum on the soft integument of venter there are some thirty-nine pairs of spines and a small scutum between the ventral scutum and the lateral border of the body. Stigmal pore level with coxa iii, peritreme reaching to anterior edge of coxa ii. Legs short and stout, chaetotaxy similar to the female except that the pair of spurs on distal end of tarsi ii and iii are stouter than those of the female.

Dorsal scutum distinctly shouldered, with a wide uncovered portion of the dorsum flanking it laterally and posteriorly. Spines on dorsal scutum shorter and thinner than those on the venter, but possessing a number of longer spines on the posterior rim.

Host. A water rat (*Otomys irroratus* Brants).

Locality. Caxton, Bathurst, South Africa. 15 May 1939.

Measurements. ♀ 0·92 × 0·71 mm., ♂ 0·78 × 0·6 mm.

The male was taken on a water rat (*Otomys* sp.) at Groenpunt, South Africa, 6 December 1938. A female has also been taken on a Cape serut shrew (*Mysorex varius* Smuts) at Caxton, Bathurst, South Africa. 31 May 1939.

In this species it will be noticed that the tooth-like process, like an inverted Λ on distal edge of the coxae, is also present on the trochanter. This process is also present on the dorsal surface of these segments. Ewing (1939) refers to this process in the Tarsoneminae as 'the articulating condyle of coxa' and the socket into which it fits as 'the acetabular process of femur'. It serves the purpose of a ball-and-socket joint, but has only been noticed on the coxae of other mites.

Genus **Ophionyssus** Megnin, 1884

Ophionyssus serpentium Hirst, 1915.

The venter of the male (Fig. 7) shows the sterno-ventral and anal scuta to be widely separated. Anterior margin of sterno-ventral scutum almost straight, level with anterior third of coxa ii, lateral edge of scutum having an irregular outline, scutum extending to posterior of coxa iv.

At the antero-lateral corner of the scutum is a small, stout, tooth-like spur (Fig. 9). The genital pore is situated on anterior margin of the scutum and is flanked by first pair of sternal spines. Second pair of sternal spines placed behind first lateral concavity, third pair of spines level with anterior third of coxa iii, one spine being off the scutum. Metasternal pair of spines situated posterior to middle of coxa iii and flanking the scutum. The genital pair of spines level with middle of coxa iv, and midway between the scutum and coxa.

Anal scutum pear-shaped, placed some distance in from the posterior end of the body, posterior end of pore on level with middle line of scutum. Paired anal spines level with middle of pore, unpaired spine midway between pore and apex of scutum.

The soft integument of the venter between the ventral and anal scuta is furnished with ten pairs of spines. Posterior to the paired anal spines and flanking the anal scutum is a pair of spines. Towards the lateral edges of the venter there are some seven or eight pairs of spines.

Legs i and iv longer than the others, legs i stouter than remainder. Chaetotaxy of legs normal.

Stigmal pore between coxae iii and iv, peritreme short, only reaching to coxa ii.

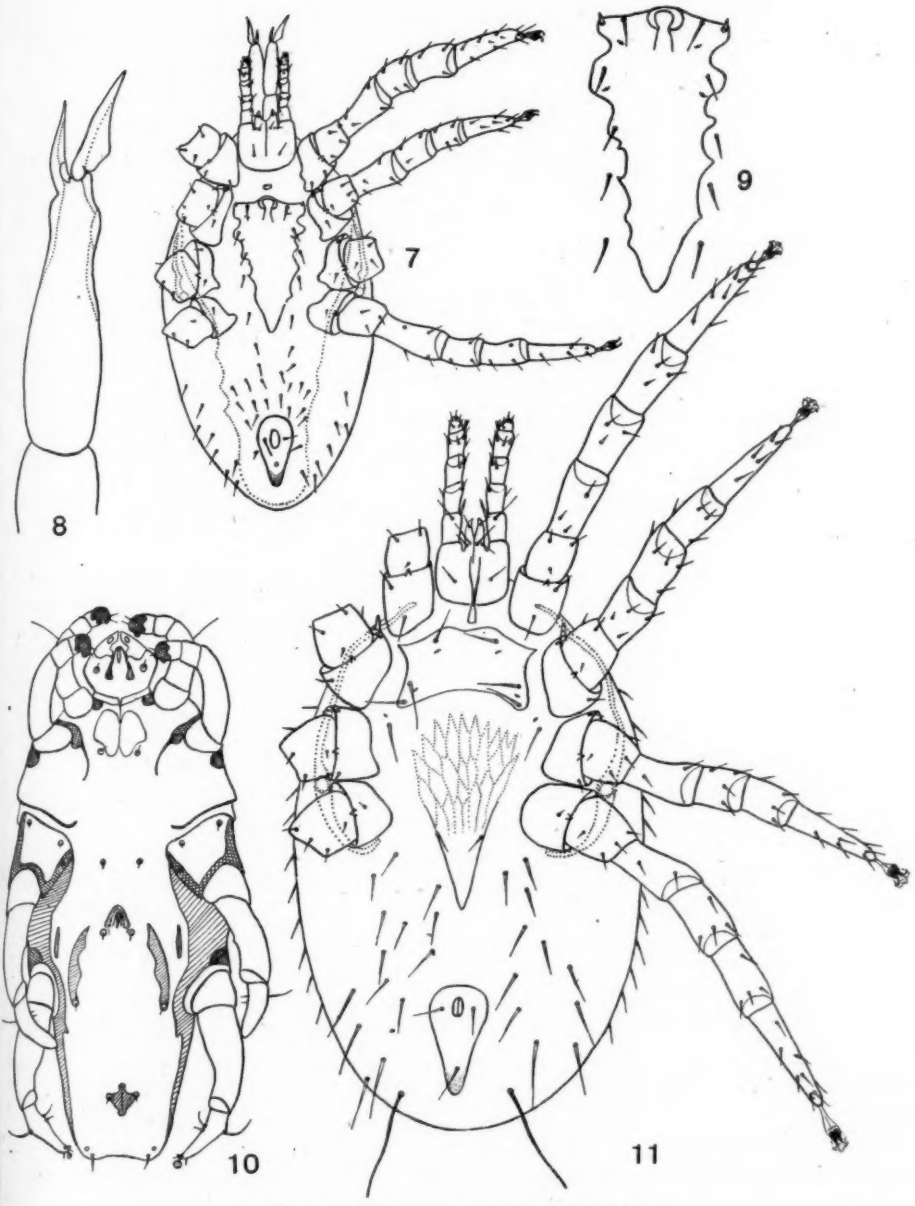
The left chela of the male (Fig. 8) shows the fixed finger to be a stout, curved, tooth-like process, with the movable finger a much stouter, blade-like organ. Dorsal scutum narrow, only covering dorsum down its centre, leaving a wide uncovered area laterally. Spines on dorsal scutum few, slender, not as long as those on venter. Spines on uncovered dorsum longer.

Host. A rainbow boa (*Epicrates cenchris* L.).

Locality. Manchester, England. 1 June 1940.

Measurement. ♂ 0·57 × 0·31 mm. (without capitulum).

Hirst's specimens were taken on a Couper's snake (*Thamnophis ordinoïdes ordinoides* B. & G.) at the London Zoo.



Figs. 7–9. *Ophionyssus serpentium* Hirst, 1915.

Fig. 7. Male venter. Fig. 8. Chelicera of male. Fig. 9. Sterno-ventral scutum of male.

Fig. 10. *Listrophoroides dasymys* n.sp. Male venter.

Fig. 11. *Liponyssus dendropicos* n.sp. Female venter.

Genus **Listrophoroides** Hirst, 1923

Listrophoroides dasymys n.sp.

The male venter (Fig. 10) shows the body to be about three times as long as broad, flattened dorso-ventrally. Legs i and ii placed on the anterior end of the body, legs iii are situated just anterior to the middle, and legs iv posterior to the middle. Conforming to the generic type in legs i and ii being more slender than the others, legs iv being the stoutest. Chaetotaxy of legs sparse, two small spines on tarsi i and ii, with a long spine on the following segment of legs ii. Spines on tarsi iii and iv almost identical. Tarsi of legs i and ii forming a claw-like grasping organ by which the mite attaches itself to the hairs of the host. Tarsi iii and iv possessing a small, pedunculated pulvillus. Ventral spines few, short, about ten pairs. One pair on capitulum, one pair level with coxa ii, a pair level with coxa iii, a pair posterior to and flanking the penis, a pair on the posterior tip of scutum flanking coxa iv, one pair on the lateral corners of anal scutum and a terminal pair at posterior end of body. On coxa iii there are three small spines.

This species is readily distinguishable from the other known species by the arrangement and shape of the chitinized scuta. Female unknown.

Host. A swamp rat (*Dasymys* sp.).

Locality. Kisanje, Kigezi, Uganda. 5 July 1940.

Measurement. ♂ 0·4 × 0·18 mm. (including capitulum).

Genus **Liponyssus** Kolenati, 1858

Liponyssus dendropicos n.sp.

The female venter (Fig. 11) shows the sternal scutum to be broader than long, the anterior margin level with middle of coxa i, slightly concave medially. The anterior margin then diverges outward and backward to the antero-lateral corners slightly behind anterior edge of coxa ii. Lateral edge of scutum concave, postero-lateral corners rounded, level with posterior edge of coxa ii. Posterior margin of scutum concave.

First pair of sternal spines anterior to antero-lateral corners, but not on anterior margin of scutum. Second pair of sternal spines placed well back towards the third pair of spines, which are situated in the postero-lateral corners. Sternal scutum reticulated and provided with the usual two pairs of pores, the first pair posterior to the first pair of spines, second pair of pores between second and third pairs of spines.

Meta-sternal scutum absent, but the spines are level with anterior half of coxa iii, and the third pair of pores are placed anterior to the spines, level with anterior edge of coxa iii. Genito-ventral scutum wedge-shaped, extending from level of meta-sternal spines where it tapers towards its posterior tip about one-third of the distance between coxa iv and end of body. The genito-ventral scutum is reticulated and bears the genital pair of spines level with coxa iv. Anal scutum elongate, pear-shaped, its anterior edge about midway between coxa iv and end of body. Anal pore in anterior third of scutum, paired anal spines level with middle of pore, the unpaired anal spine in posterior third of scutum.

On the soft integument of venter there are some fifteen pairs of spines, the posterior-most pair of spines being almost twice the length of the others. A number of spines are carried on the lateral edge of the body. Legs short, sub-equal, chaetotaxy normal except

that the anterior edge of coxa ii bears a strong, tooth-like process. First free segment of palp is furnished with a long, tooth-like process on the inner distal edge.

Stigmal pore between coxae iii and iv, peritreme reaching to middle of coxa i. Posterior to the pore there is a continuation of the peritremal scutum round coxa iv to its posterior edge.

Dorsal scutum completely covering dorsum, spines few. Male unknown.

Host. A woodpecker (*Dendropicos fuscescens lapidus*).

Locality. Kinkizi, Kigezi, Uganda. 5 August 1940.

Measurement. ♀ 0·85 × 0·53 mm. (without capitulum).

To Dr R. R. Parker, Rocky Mountain Laboratory, Hamilton, Montana, U.S.A., Dr G. Theiler and Mr P. J. du Toit, Zoological Survey Committee, Onderstepoort, South Africa, Mr G. H. E. Hopkins, Senior Entomologist (Medical) Kampala, Uganda, and Mr Gerald T. Iles, Belle Vue Zoological Gardens, Manchester, I wish to acknowledge my thanks for the specimens described in this paper.

The holotype and allotype slides are being placed in the collection of the British Museum (Nat. Hist.).

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FERTILITY IN THE ARGASID TICK, *ORNITHODORUS MOUBATA*, MURRAY

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Introduction

The experiments described here attempt to define the best routine method to follow in breeding the tick, *Ornithodorus moubata*, for tests with contact insecticides. Previously the viability of eggs was seen to be rather variable, and for some weeks was so inconveniently low (Robinson, 1942) that the present work seemed highly desirable.

As a rule female ticks being studied were each kept in a small dish of sand in a constant temperature room at 28° C. and 50% relative humidity. Females selected for experiment were allowed to emerge from the previous nymphal stage in separate dishes, and were generally used within a month after emergence. The general method was to study two groups of ticks which had exactly the same treatment, except for a difference in one factor which was thought to have an effect on fertility.

The experiments were for the most part utilitarian for the purpose of conserving the stock, and were not intended to cover comprehensively all aspects of fertility. For example, the effect of the age of the adult tick was not investigated because it was not intended to keep stock females for any considerable time. (Davis (1941) states that male fertility in *O. turicata* does not deteriorate with age.) Again, temperature experiments were confined to fertility of the eggs, and did not include assay of egg production since, even in the event of the egg-laying optimum temperature differing from the developmental optimum, it was not intended to have the females and their eggs in different incubators.

Insemination

(*a*) *The efficacy of one insemination*

Jobling (1925) remarks that 'the percentage of eggs capable of development gradually declines to the fifth batch, and it may happen that none develop at all in the sixth batch'. He further says that this decline is due to senescence of the female, and not to absence of spermatozoa. The implication is that the females he used were inseminated only once. (Confirmed by private communication.)

In the raising of the present stock of ticks, therefore, it was at first assumed that one copulation would provide the female with enough sperm for two or three acts of oviposition. Since the second batches of eggs showed some infertility, the effect of the constant presence of a male with each female was tested on one group, and a parallel group was observed, each female of which had been mated only once. The females were each isolated in small dishes lined with filter paper. The results are recorded in Table 1.

The numbers of females were reduced for second and third batches of eggs owing to deaths and to failure to oviposit in some cases over a period of 5 weeks from the time of feeding. About 1 month intervals were allowed between feedings.

From Table 1 it is obvious that fertility is lower in the second and third batches of eggs than in the first batch of both groups of females. Also the decrease in fertility is significantly greater in the eggs laid by females mated only once. The presence of the

Table 1. *Effect of the presence of the male with the female* Ornithodorus moubata *on the fertility of the first three batches of eggs*

Condition of ♀♀	1st oviposition No. of ♀♀	1st oviposition Eggs Total	1st oviposition Eggs % infertile	2nd oviposition No. of ♀♀	2nd oviposition Eggs Total	2nd oviposition Eggs % infertile	3rd oviposition No. of ♀♀	3rd oviposition Eggs Total	3rd oviposition Eggs % infertile
Attended by ♂	30	5298	5 ± 0·91	24	3739	15·5 ± 0·67	7	895	13 ± 1·48
Mated once	15	2387	3 ± 1·34	14	2150	30·5 ± 0·88	6	539	49 ± 1·91
Totals	45	7685	4·5	38	5889	21·0	13	1434	27·0

Table 2. *The effect on fertility of single and multiple mating in* Ornithodorus moubata

Condition of ♀♀	No. of ♀♀	1st oviposition No. of eggs	1st oviposition % infertile	2nd oviposition No. of eggs	2nd oviposition % infertile	3rd oviposition No. of eggs	3rd oviposition % infertile
Mated before each feed	8	1134	0	1291	11 ± 1·03	1126 (from 6 ♀♀)	28·5 ± 1·42
Mated once	9	1157	1·6 ± 0·19	1824	20 ± 0·87	1871	51·0 ± 1·11
Totals	17	2291	0·8	3115	16·4	2997	42·7

male apparently increases the fertility of the second and third batches of eggs, but the fact that 100% fertility was not reached points to the existence of other factors of influence. The apparent senescence mentioned by Jobling is fully confirmed.

Experiments were done in a similar way, except that where attendance of the male had been relied upon in the previous tests, successful insemination in each female was now actually observed. Thus failure of the male to copulate was eliminated as a source of error in assessing the importance of fresh mating for each batch of eggs. The females were allowed to lay their eggs in sand, and the results are in Table 2.

The same conclusions are to be drawn from Table 2 as from Table 1, namely, that fertility decreases from the first to the third batch of eggs, and that this decrease is significantly larger in the batches from females mated only once than in batches from females mated before each feed.

(*b*) *Fertility and the interval between mating and feeding*

Two groups of females were observed; all individuals were of the same age within a week. One group was mated forthwith, and the other group was mated after 8 weeks had elapsed, all ticks being kept in different dishes of sand in the interval. All ticks

were fed after the 8 weeks had passed and their egg-production noted. The results are seen in Table 3.

The difference in column 5 is significant and indicates that sterility of the eggs increases with the interval between mating and feeding. The age of the eggs in the ovary being the same in the two groups of ticks compared, the variable factor was the interval between the entry of the sperm into the female and laying of eggs. This may imply that the spermatozoa tend to deteriorate in the oviducts. Christophers (1906), Nuttall & Warburton (1908), and Samson (1909*a*) have observed sperms in the ovary of *Ornithodorus moubata*, but the present results suggest that fertilization of a batch of eggs does not take place until the ovary is in full production under the influence of a blood meal. Another possibility is that fertilization can take place as soon as the sperms enter the ovary, but if the female remains quiescent for a considerable period, the eggs in her ovary will have a better chance of survival if sperms are withheld from them until near the time of the blood meal.

Table 3. *Influence of the interval between mating and feeding on fertility in* Ornithodorus moubata

Mating	No. of ♀♀	No. of eggs	No. infertile	% infertile and standard error
1 day before feeding	8	1638	191	11·7 ±0·91
8 weeks before feeding	6	862	229	26·5 ±1·25
	Totals	2500	420	16·0

The organ of Géné

This organ plays an important part in egg laying, and paints each egg with a sticky secretion as soon as it comes out of the vulva (Samson, 1909*b*). Bertkau (1881) states that if the organ of Géné be pricked and put out of action in *Ixodes ricinus*, then eggs laid subsequently shrivel and fail to develop.

The mouth of the organ of Géné on three female ticks due to lay eggs was stopped with rubber solution. Two of the ticks managed to evert the organ and laid normal batches of eggs which were 90% fertile. The remaining female could not evert the organ and all her eggs were infertile, and shrivelled on the same day as they were laid. The skin of rubber was removed and the female was allowed to lay a second batch of eggs which were fertile to a normal degree. Another female was observed which had its organ permanently everted ever since emergence from the nymphal skin. The organ appeared hard at the extremities and was not sticky as in healthy ticks. All the eggs of the first batch soon shrivelled and did not develop. In the course of stock raising it has been seen that ticks with faulty ovipositing organs lay 100% sterile eggs.

Temperature and humidity

Brett (1939) has shown how tolerant *Ornithodorus moubata* is of wide ranges of relative humidity. Full fertility is possible at 50% saturation or more, and failure of eggs becomes serious only below 30% relative humidity.

In these experiments the eggs of this tick were found to have a similarly large tolerance of temperature range. The eggs were put in little dishes confined in jars over saturated ammonium chloride solution giving a relative humidity of about 80%. The temperature

varied not more than 0·5° C. from the average in the case of each dish of eggs. The percentage of larvae developed was taken as the index of fertility. From Table 4 it can be seen that eggs develop equally well throughout the range 22–32° C. Somewhere between 32 and 34° C. there is a very sharp drop in fertility rate and no eggs develop at all at 34·5° C.

This sharp transition from survival to death of insects over a small range of temperature has been noticed by Mellanby (1932) who exposed lice and fleas to various temperatures for periods of 1 and 24 hr.

For the experiment the eggs were taken at random from a group composed of batches from several females, so that the possibility of inherent sterility prejudicing the results was eliminated. It was necessary to run the experiment twice to include all the temperatures, and column 1 shows which temperatures were included in each of the runs. Although eggs in Exp. 1 had a different origin to those in Exp. 2, it is thought that the results are homogeneous.

In egg-production experiments with this tick, Cunliffe (1921) used only three temperatures, 22, 30 and 37° C. The latter temperature proved too high for normal repro-

Table 4. *Fertility of eggs of* Ornithodorus moubata *at various temperatures (80% relative humidity)*

Exp.	Temp. °C.	No. of eggs	No. of larvae	Fertility rate
1	34·5	243	0	0
2	32·3	423	325	77
2	31·0	433	310	72
1	30·5	365	254	69
2	29·5	348	272	78
2	26·6	383	299	78
1	26·5	259	194	75
2	22·5	350	261	75

duction, which might be expected from the present results, but this author found that 30° C. gave a lower fertility rate than 22° C. This is not substantiated by the present experiments in which the fertility rate varied little within the range 22–32° C. The rapidity of development at 30° C. makes it usually preferable to lower ranges if a large stock is required.

In contrast to eggs of this species, eggs of *O. coniceps* are very sensitive to variations of both temperature and humidity (Bodenheimer, 1934). Below 80% relative humidity no development takes place at all, and above or below the optimum temperature the fertility rate falls off rapidly. In these respects *O. moubata* is more like *Argas persicus* which has a similarly large tolerance of climatic changes (Bodenheimer, 1934).

The rate of egg laying

Both the pre-oviposition period (time between feeding and egg laying) and the total number of eggs laid are affected by the physical environment of the engorged females. A series of mated and engorged females was kept in sand about ½ cm. deep, and a similar series was kept on filter paper. This experiment was prompted by a desire to simplify collection of eggs. It is much simpler to remove eggs from filter paper than to collect them from sand, but casual observation suggested that egg production was greater if the ticks were allowed to burrow in sand. Each female was confined by herself in a small dish. The pre-oviposition period varied according to Table 5.

Absence of sand retards the onset of egg laying to a significant degree (2 days retardation in 10 days). The number of eggs laid varied according to Table 6.

The highly significant effect of the environment on egg production is seen in column 4. The females in sand had about two-thirds of their body exposed to the atmosphere, so that any difference in micro-climate operating on the individuals of the two series was negligible. The relative humidity of the atmosphere in the desiccator used was 50%, and in view of the tolerance of this tick to wide ranges of relative humidity (Brett, 1939) it seems probable that the differences in egg production are accounted for purely by differences in mechanical nature of the environment. Column 3, Table 6, shows how egg production is curtailed by the open environment.

Table 5. *Variation of the pre-oviposition period with the physical environment of* Ornithodorus moubata

Pre-oviposition period in days ...	7	8	9	10	11	13	14	15	16	18	19	21
No. of females in sand	1	5	2	7	7	1	1	.	.	.	.	1
No. of females on filter paper ...	.	.	1	2	10	9	1	1	1	1	.	.

Average pre-oviposition period and standard error in days ... ...
Sand 10·4 ±0·56
Filter paper 12·5 ±0·46
Significance value (mean diff./s.e. diff.) = 3·1·

Table 6. *Variation of the number of eggs laid by females of* Ornithodorus moubata *in sand and on filter paper*

Environment	No. of females	Duration of egg laying with standard error	Average no. of eggs per individual with standard error
Sand	25	9·5 ±0·75	143 ±9·5
Filter paper	27	6·1 ±0·65	39·6 ±3·3

Table 7. *Variation of the pre-oviposition period with the physical environment of* Ornithodorus moubata*: second oviposition*

Environment			Pre-oviposition period in days							Present average		Previous average	
Previous	Present		7	8	9	11	12	13	14				
Sand	Sand	No. of ♀♀	7	3	.	.	.	.	1	7·9 ±0·65	8·3 ±0·36	10·7	11·4
Filter paper	Sand		4	6	1	.	1	.	1	8·5 ±0·60		11·8	
Sand	Filter paper		.	5	1	2	.	.	2	9·9 ±0·81	9·5 ±0·65	10·1	11·5
Filter paper	Filter paper		3	2	4	1	.	1	1	9·2 ±0·67		12·7	

The same ticks used in the above experiment were used again in a more critical test. The relative humidity was raised to 90% in order to diminish the slight difference which might possibly occur in the microclimates of the two environments tested. Each group (those on sand and those on filter paper) was divided into two lots which the experimental records showed had been of roughly equal productivity (Table 8, col. 6). One lot of each group was transposed to the alternative environment, all were fed, and the egg production was noted. Each lot of ticks was weighed before and after feeding (Table 8, col. 5), so that the average uptake of blood in each lot was known. Each tick was marked so that it would be identified after feeding, and its behaviour in this experiment compared with its previous behaviour. In this way it was hoped to assess the influence of the environment as distinct from the degree of feeding, and also in relation to egg production in the previous oviposition. The results are seen in Tables 7 and 8.

From Table 7 it is seen that the second pre-oviposition period is 2–3 days shorter than the first, and this may in part account for the lack of statistical significance in the difference of the effects produced by the two environments. The time of onset of egg laying on sand proved 1·2 days previous to that on filter paper, so that the results have the same direction as those for the first oviposition.

In Table 8, col. 4, it is seen that the duration of laying of second batches is identical in the two environments. Nevertheless an environment of sand still proves to be much more productive of eggs than filter paper, as in the first batches of eggs (col. 7). The ticks on filter paper imbibed about 3·5% more blood than those in sand (col. 5), so that differences in feeding can be ruled out as the prime cause of the difference in egg production. (Jobling (1925) has shown that females fed for only 10 min. lay about a third as many eggs as those fed for the normal half-hour or so. This, of course, would mean the imbibition of very much less blood than in a normal feed.) The previous history of the two groups was almost identical (col. 6). Each of the lots previously on sand laid an average of about 140 eggs, and those previously on filter paper about 40 eggs. These

Table 8. *The number of eggs in second batches laid by* Ornithodorus moubata *in sand and on filter paper*

Environment		No. of ♀♀	Duration of egg laying with standard error		Uptake of blood in mg./tick		Average no. of eggs per individual with standard error		
Previous	Present						Previous	Present	
Sand	Sand	11	12·9 ± 1·1	12·0	116	111	137	193 ± 11·4	170 ± 7·8
Filter paper	Sand	13	11·2 ± 0·9		107		40	152 ± 10·6	
Sand	Filter paper	10	13·8 ± 1·5	12·0	125	115	143	73 ± 6·3	73 ± 4·2
Filter paper	Filter paper	12	10·5 ± 1·4		106		40	73 ± 5·7	

performances showed little effect on productivity of the second oviposition over which the environment had still the largest influence. As stated above, about equal numbers from each environment were transposed for the second oviposition which should therefore not be affected by previous egg production since the latter was almost identical in the two groups.

The important conclusion is that this tick lays over twice the number of eggs in sand as it will on filter paper, and it should therefore be given the former environment for maximum egg production.

CONCLUSIONS

The requirements under laboratory conditions for maximum egg production in the tick, *Ornithodorus moubata*, are as follows: a new insemination of each female for each batch of eggs, which should take place a day or two before the blood meal; a relative humidity of 50% or more should prevail in the breeding chamber and a temperature of 30° C. can be considered as definitely safe and speedy, although the eggs are tolerant of the range 25–32° C.; females with faulty ovipositing organs should be discarded as useless; the females should be put in about 3 or 4 mm. of sand for egg laying.

The present study has shown great individual variability of reproductive potentiality. The female usually lays approximately 170 eggs about a fortnight after a blood meal at 28° C., yet it is not unusual for egg laying to be delayed 2 or more months or for the female to die before any eggs are laid. Again, occasionally females have been noticed

to lay up to twenty fertile eggs without having fed in the adult state. The females vary in size considerably and this affects the number of eggs they lay. Also the number of eggs laid by females of the same size varies from 50 to 250 (average 170). Many females die a day or two after their first blood meal, sometimes through burst guts, and many die after laying their first batch of eggs.

In spite of these variations in fecundity it has been fairly easy to determine the optimum breeding conditions for this tick for the purpose of stock rearing in the laboratory. No particular condition of culture was discovered which was causing the high sterility of the eggs, and the stock gradually came back to its normal level of fertility. In the event of failure of the eggs recurring, the ascertained optimum conditions would give the stock the best chance of survival. The sterility was possibly due to a harmful genetical factor which has been superseded by the normal factor for the time being.

Summary

The fertility rate of eggs of the tick, *Ornithodorus moubata*, was investigated with the following results:

(1) Although three batches of eggs can be laid after a single mating, fertility is considerably improved if a fresh mating take place before each oviposition.

(2) Fertility of the eggs decreases with the interval between mating and oviposition (the latter takes place usually only after a blood meal).

(3) If the organ of Géné is faulty (permanently everted) none of the eggs will develop.

(4) Eggs have a wide tolerance of temperature, and show no alteration in fertility rate from 22 to 32° C. At 34° C. no larvae are produced at all.

(5) Females lay over twice as many eggs in sand as they do on a flat surface such as filter paper.

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THE SIZES OF VIRUSES AND THE METHODS EMPLOYED IN THEIR ESTIMATION

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I. Introduction

At present the exact size of no virus is known. The numerous discordant results encountered in the literature dealing with the filterability, size and visibility of viruses are probably due to inadequate experimentation, careless thinking, prejudice, imperfect experimental methods and the difficult nature of the problems. (Rivers, 1932)

The above comments on our knowledge of the sizes of viruses were made just ten years ago with, it must be admitted, a good deal of justification. In this paper we have endeavoured to give a detailed review of the position regarding the measurement of virus particles as it stands to-day. A glance at the tables of sizes, which we have compiled from all the modern data available, indicates that numerous discordant results still exist although the great improvements in technique have somewhat modified these.

Much work has been done on the sedimentation and diffusion rates of viruses, and from these measurements their molecular weights and particle sizes have been calculated. Such indirect methods of computation involve the use of certain assumptions, and the criticism has been made that some at least of these assumptions are very unlikely to be true (J. H. Smith, 1940). Nevertheless, when a particular virus has been investigated by a number of indirect methods of particle measurement, the general agreement in the results obtained is fairly good. Luckily we now have in the electron microscope a direct method for measuring the sizes and shapes of virus particles, and this can be used to check the

results achieved by more indirect means. Such confirmation has been obtained already in the cases of three viruses, those of tobacco mosaic, tomato bushy stunt, and vaccinia.

If we do not yet know the exact size of any virus we now have a considerable amount of information on the sizes of the three viruses mentioned above, and these are discussed in detail in § X.

A. METHODS OF SIZE DETERMINATION

II. General survey of methods

(1) *Ultra-violet light and electron microscopy*

The most direct method of finding the size of a virus is by ultra-violet light or the electron microscope. The wave-length of ultra-violet light which can be used with a quartz objective not being much below 2000 A., only the larger viruses of 100 mμ and above can be measured by this means. The wave-lengths of electrons of about 60 kV. energy being 0·05 A., the theoretical limit of resolution of the electron microscope is well beyond that necessary for the accurate measurement of even the smallest viruses. However, with present instruments the resolution is of the order of 5 mμ, and as a result of the blurring of the image, due partly to insufficient resolving power and partly to the diminished opacity of the object near its boundary, the accuracy of a measurement of the diameter of a small virus is at present not better than about 5 mμ. Photometry of the photographic images, and some improvement of resolution, should eventually make this method accurate for small viruses. In its present form it is capable of giving accurate measurements of the sizes of the larger viruses, though up to the present not many viruses have been examined.

The great merit of the use of the electron microscope, assuming its resolution adequate for the virus under investigation, is that the measurement of size is direct and is not subject to error due to the necessity of interpreting the measurements by theoretical formulae of the validity of which it is difficult to be completely certain.

At the present time, when the accuracy of the electron microscope is hardly great enough for the measurement of the smallest viruses, its principal use when these are being investigated is the determination of shape. Other methods, particularly diffusion and sedimentation methods, are worked out primarily for spherical particles and require modification for particles markedly aspherical. Even at its present level of precision the electron microscope is able to show, for example, that the ratio of length to breadth of tobacco necrosis virus or tomato bushy stunt virus probably does not exceed 3 : 2, and thus justifies the use of equations derived for spherical particles when these viruses are being investigated by diffusion or sedimentation experiments. Again, while other methods earlier indicated that tobacco mosaic virus was rod-shaped, the electron microscope gives a more accurate estimate of the axial ratio than less direct methods, and enables the distribution of length to be determined. Moreover, by this means Luria & Anderson (1942) have shown that certain bacteriophages have a shape which is not readily amenable to the usual mathematical treatment of sedimentation, diffusion, etc. The bacteriophages *Coli* P.C., *Coli* P28, and *Staphylococcus* 3K have been shown to consist of an almost spherical 'head' portion with a long thin 'tail'.

Viruses examined by the electron microscope have necessarily to be prepared in thin dried films and exposed to a high vacuum. The size measured is therefore of the dried virus, which may differ from the size of the virus in aqueous solution. Further, the act of

taking an exposure necessarily results in the virus being bombarded by electrons, which cause it to be inactivated, supposing it not to have been already inactivated by the drying in vacuo. There is no reason, however, to suppose that this last effect leads to any error in the size determination; prolonged exposures, however, have been shown by Pfankuch & Kausche (1940) to result in the disintegration and distortion of bacteriophage particles.

(2) *X-ray diffraction*

This method is only applicable to viruses obtainable in a crystalline or semi-crystalline form and has so far been successfully applied only to tomato bushy stunt virus and to the tobacco mosaic group of viruses. In a bushy stunt virus crystal or a tobacco mosaic virus gel the virus particles are arranged in a regular array or pattern. This array serves as a diffraction grating to a beam of X-rays, and measurement of the angles at which the X-rays of known wave-length are diffracted enables the plan of the pattern and the repeat interval to be established. Thus in the case of wet crystals of bushy stunt virus, it has been found that the pattern is a body-centred cubic lattice of edge 394 A. In other words, if one imagines the crystal to be built up out of cubes of this size, then at the centre of each cube, and at every corner, there is either one virus particle, or an identical group of virus particles. There will thus be two particles, or groups, per cube of edge 394 A., and knowing the density of the crystal the molecular weight of the particle, or group, can be calculated. In the absence of information to the contrary it has been assumed that the element in the lattice is a single particle and not a group.

In both tobacco mosaic and bushy stunt viruses the crystals or gels may be formed with a range of hydrations, the virus particles remaining equally spaced but increasing the spacing as the degree of hydration increases. The spacing in the case of the *dried* crystal or gel is likely to be most significant, since it should be related to the size of the dry virus particle. It is possible that the spacing in the wet crystals or wet gel may bear a similar relation to the size of the (hydrated) virus particle in solution, but it is hardly safe to take this for granted.

The X-ray diffraction method is of limited application, but its great merit is that it gives accurate information about the sizes of the smallest viruses. In fact, the accuracy of measurement is in principle greater as the virus becomes smaller. It is stated that the separation of the central axes of virus particles in a dry gel of tobacco mosaic virus can be measured with an accuracy of 0·2%, while, judging from the mutual consistency of estimates of lattice constant derived from reflexions from different planes of the crystal, the measurement in the case of bushy stunt virus should be correct to about 1%.

The virus will be completely inactivated by the exposure to the X-rays necessary to obtain the photograph. There is no reason, however, to anticipate any error from this cause.

(3) *Sedimentation and diffusion*

A more detailed account of these important methods is given later. The experiments consist of determining s_{20} and D_{20}, the sedimentation and diffusion constant respectively, the former being the rate of sedimentation in cm./sec. under a centrifugal acceleration of 1 dyne/g., and the latter being the rate of transference of virus across unit area under a unit concentration gradient. Experimental values are conventionally corrected to water at 20° C.

The sedimentation constant is determined by measuring the rate of sedimentation in the ultracentrifuge. If an optical method is used for determining the position of the boundary, the diffusion constant can in principle be determined at the same time by observing the increasing blurring of the boundary as sedimentation proceeds. This method is not likely to be accurate with viruses, but providing slow speeds are used the method of sedimentation equilibrium can be used to give an estimate of s/D and hence of D_{20} and s_{20}. Alternatively, the diffusion constant can be determined without the use of the centrifuge.

For a spherical, non-hydrated virus, the partial specific volume of which is known, measurement of either s_{20} or D_{20} alone gives the size of the virus. If determinations of both s_{20} and D_{20} are available, they suffice to determine the degree of hydration, supposing shape to be known, or to determine the shape, supposing degree of hydration to be known. Shape and hydration enter into the equations for diffusion constant and sedimentation constant because the addition of water to a particle increases its size and therefore its frictional resistance, while a non-spherical particle suffers more frictional resistance than a sphere of the same volume. It happens, however, that for an axial ratio not exceeding 2 : 1, the friction suffered by a non-spherical particle is very little different from that suffered by a sphere of the same volume. It follows therefore that if, by such means as electron microscopy, X-ray diffraction, or crystallographic classification, one obtains an indication that the virus is not *markedly* aspherical, then its effective radius and molecular weight and degree of hydration may be obtained from sedimentation and diffusion data even though its *exact* shape is not known.

(4) *Filtration*

The method of determining the size of a virus by filtration through collodion membranes of graded porosity depends upon (*a*) determining the pore size of each membrane used in terms of a conventional measure known as the 'average pore diameter' (A.P.D.), (*b*) relating for the type of membrane used the A.P.D. to the size of particle just stopped by the membrane. This is the method used by Elford, and in § IV we give a revised assessment of the relation between A.P.D. and particle size for nearly spherical particles. There is as yet no reliable knowledge of the relation between A.P.D. and particle size in the case of markedly aspherical particles.

Filtration measurements on viruses are usually made by filtration end-point determinations using infectivity tests on the filtrate. It is evident that in the event of polydispersity the size determined will not be the average size but the size of the smallest component. In the case of a preparation of high activity the presence of even a minute amount of a smaller component will result in the end-point determined being that of this component. Sedimentation and diffusion measurements are often carried out by an optical method, and traces of smaller components will be overlooked. This is made evident by imagining, for example, that in a given preparation 99% of the particles are of a given size, and 1% of half the diameter (one-eighth the weight). Providing the infectivity of the original virus is sufficient to stand dilution a hundred times or so, the filtration end-point should be that of the small component. On the other hand, this component has only a fraction, 0·12% of the mass of the large component, and in a sedimentation experiment made by means of the absorption method it will not show up as a separate boundary.

Polydispersity should reveal itself in a gradually sloping or benched filtration curve.

Such curves are obtained with some plant viruses. Since it is possible to imagine that rod-shaped particles would give similar curves even if monodisperse, the significance of an abnormal filtration curve cannot be decided in any particular case. With tobacco mosaic virus for example, which is known to be rod-shaped, it is reasonable to ascribe the anomalies in the filtration curve to its shape. On the other hand, the virus of tobacco necrosis appears to be nearly spherical in electron microscope photographs, and it is plausible here to imagine that polydispersity accounts for the bench-shaped filtration curve.

In view of these considerations, it is only when a steeply descending filtration curve is obtained without a bench that one can infer the size of the virus with any confidence, but a non-typical curve may give an indication of polydispersity which may be overlooked by other methods.

(5) *Radiation inactivation*

This recent method is based on the fact that, when a virus is inactivated by ionizing radiation (i.e. X-rays or a radioactive radiation, but not ultra-violet light), it is possible to calculate from the amount of inactivation produced by known doses of radiation what may be called the radio-sensitive volume of the virus, i.e. the volume of that part of the virus within which energy must be absorbed from the radiation for inactivation to occur. Absorption is a highly localized phenomenon, and is sufficiently energetic for it to be tolerably certain that when energy absorption, i.e. ionization, occurs in a particular atom, the molecule or radicle of which that atom is a part suffers chemical change. The radiation method thus measures the total volume of all those molecules or radicles which are so essential to the virus that infectivity is no longer retained when any one of them suffers chemical change. In a large virus such as vaccinia a great deal of the material in the virus is not essential in this strict sense, and the radio-sensitive volume is only a very small fraction of the volume of the virus. On the other hand, a virus which crystallizes is presumably a single molecular species, and we can reasonably expect that chemical change anywhere in the molecule will cause loss of infectivity or change in the symptoms. In this event the radio-sensitive volume will be identical with the volume of one molecule of the virus, i.e. the least quantity of the nucleo-protein having the characteristic properties of the virus.

It is on this basis that the method has been proposed as a means of determining the sizes of the crystallizable plant viruses. The sizes obtained agree with published sizes obtained by filtration methods (Lea & Smith, 1942), but are somewhat lower than the sizes which in this review we conclude are the most probable sizes for the viruses in question (see § IV). This may be due either to the virus particles in solution not being single molecules but polymerized molecules, or alternatively to the structure of a virus molecule not being absolutely unique; but a certain degree of variation in structure may be possible without the virus losing its characteristic properties by which one tests its activity in the radiation experiment. The method is only applicable to viruses the particles of which we have reason to believe are single molecules. Completely misleading results will be obtained if we attempt to apply radiation methods to calculate the size of a virus which is not simply a nucleo-protein, but which is more in the nature of a single-celled organism with a diverse chemical content. With such viruses the comparison of the radio-sensitive volume with particle size may give information on the nature of the virus, but such considerations are outside the scope of this review.

(6) *Miscellaneous methods*

As it is possible to see some viruses microscopically, the number of particles in a known weight of purified virus can be found by counting under the microscope using dark-ground illumination. Moriyama (1939) has applied this method to the estimation of the size of vaccinia virus from various sources and obtains the value 0·23–0·27 μ for the dry diameter of the virus from the skin of rabbits or cattle and 0·16–0·20 μ for the virus from rabbit testis.

The weight of the smallest infectious quantity of a purified virus preparation will give a maximum value of weight and size for the particles. This technique can only be applied to animal viruses and bacteriophages as the conditions of inoculation of plants necessitate large numbers of particles. Using this method, Schramm (1939) assessed the weight of the smallest quantity of his infectious myxomatosis virus preparation as 7×10^{-16} g., a value which should exclude van Rooyen's (1937) value of 0·2–0·4 μ for the diameter of this virus, obtained by microscopical methods. Kalmanson & Bronfenbrenner (1939–40) made a *B. coli* bacteriophage preparation which was active at 6×10^{-17} g., giving a diameter, if spherical, of about 44 mμ, on the assumption of a density of 1·3. More active preparations gave a diameter of 38 mμ.

Langmuir & Schaefer (1937) have measured the thickness of layers of tobacco mosaic virus adsorbed on to conditioned films of barium stearate and egg albumin. The adsorbed layer was compared with layers of barium stearate to obtain the thickness, which was 30 mμ in the first case and 12·5 mμ in the second. The latter approximates to the value found by X-ray crystallographic methods.

Bawden & Pirie (1937) estimated the length of the particles in one of their tobacco mosaic virus preparations to be 1·4 μ from the concentration at which the virus became spontaneously birefringent. Using the value of 0·73 for the specific volume and the diameter of 15·2 mμ from the X-ray measurements, they calculated the diameter of the flat cylinder in which the virus could rotate freely in the plane of its long axis.

III. Microscopic methods

The resolving power of a perfect microscope under optimum conditions of illumination is limited by the aperture of the objective lens-condenser system, and the wave-length of light used. The maximum resolution of ordinary microscopes used with white light and homogeneous immersion systems is some 200 mμ, being given by Abbé's formula $\frac{1}{2}\lambda/NA$, where λ is the wave-length and NA the numerical aperture. The visibility of a virus is not directly related to the resolving power of the microscope but is merely a function of the contrast between the image of the virus and the background. Thus for transmitted light with a perfectly opaque (stained) virus the smallest visibility will be some 150 mμ, and with dark-ground illumination the smallest visible particle may be a small fraction of the resolving power, being limited only by the amount of light coming from the object (a function of the refractive index difference between the object and the 'solvent', its absolute size and the efficiency of the condenser) and the perfection of the dark background. Similar considerations apply when the virus is rendered apparently self-luminous by staining with fluorescent dyes (Hagemann, 1937). The apparent size of the image seen, however, will be that of a disk with a diameter of some 200–350 mμ, dependent upon the perfection of the optical system used, and the wave-length of the

illuminant. It is not surprising, therefore, that many workers have reported sizes of viruses in this range (van Rooyen, 1937; Coles, 1937, 1941; Hagemann, 1937; Taniguchi, Hosokawa, Kuga & Terada, 1935). Merling-Eisenberg (1938), Eisenberg-Merling (1941) has applied a photometric technique to the dark-ground images of bacteriophages and obtains the sizes of a *B. coli* bacteriophage and a *Staphylococcus* bacteriophage as 25 and 60 mμ respectively. Details of the measurements are not given and they would appear to necessitate assumptions regarding the refractive index of the bacteriophage particles.

The use of ultra-violet light of suitable wave-length increases the resolving power of microscopes about twice. Barnard (1925) has claimed a maximum resolution of 75 mμ for his equipment. He obtains the absolute sizes of the particles by comparison with *Chromobacterium prodigiosum* which is used as a standard of size. Interpretation of results is further complicated by purely photographic considerations such as irradiation, contrast and halation.

As the resolving power of microscopes is barely sufficient to resolve the larger viruses, it is evident that great reliance cannot be put on the measurements obtained by this means. Moreover, many authors have failed to give conclusive evidence that the particles described were actually virus particles, a serious point especially in cases where stains have been used.

IV. FILTRATION

Bechhold in 1907 was one of the earliest workers to prepare ultrafilter membranes, using gels of varied solid content so as to obtain filters with different pore sizes. The gels used were of gelatin in water or collodion in acetic acid, and the latter have been used extensively by Bechhold and his co-workers. The porosity of these membranes is measured in two ways: (*a*) by measuring the air pressure necessary to force air bubbles through the membranes into water and applying a surface-tension formula; (*b*) by measuring the rate at which water flows through the membrane under known pressure, and applying Poiseuille's viscosity law, assuming that the structure of the membrane has certain regularities. This latter method, which is generally used, has been criticized by Einstein & Mühesam (1923) and Bechhold & Villa (1926), and obviously gives rather smaller values than the former method which should give the size of the largest single pore. In order to apply surface-tension data to fine filters, liquids having small interfacial tensions in respect to water have been used, and evidence has been given to show that the range of pore sizes in a single filter is large and that the actual length of the pores may be 5–10 times the thickness of the membrane (Bechhold, 1908; Bechhold, Schlesinger & Silbereisen, 1931).

Elford (1931) introduced a new type of collodion membrane prepared in a carefully standardized manner and produced a fairly strong membrane for which great uniformity and reproducibility have been claimed. Calibration of these 'gradocol' membranes is carried out essentially in the same way as the Bechhold filters by applying the Poiseuille viscosity formula and obtaining a value for the A.P.D. A certain amount of speculation has been advanced in support of the assumptions involved in this calibration (Elford & Ferry, 1935; Elford, 1933), but is based on rather inconclusive evidence. Grabar (1938) has produced photomicrographs of the more porous gradocol membranes, and they show considerably more heterogeneity than one would be led to expect. The structure of the finer membranes is not known.

It seems from the agreement of the results obtained by various workers that the gradocol membranes are fairly uniform in structure and that two membranes prepared under similar conditions and having the same A.P.D. will behave in essentially the same manner. Some of the difficulties in obtaining uniform and reproducible membranes have been discussed by MacClement & J. H. Smith (1932) and in a detailed investigation by K. M. Smith & Doncaster (1935).

The actual size of the particle retained by a membrane of given A.P.D. has been investigated by Elford (1933), Elford & Ferry (1935) and Bauer & Hughes (1934–5). Using particles, the sizes of which were thought to be accurately known, these authors deduced a purely empirical relationship between size of particle retained and A.P.D.:

A.P.D. mμ	Ratio of particle size to A.P.D.
10– 100	0·33–0·5
100– 500	0·5 –0·75
500–1000	0·75–1·0

In view of the extensive use of this method for measuring the size of viruses, we have reinvestigated the above calibration factors in the light of more recent knowledge, and have found them to be incorrect and almost uniformly low. We have also deduced the calibration factors which apply over the range of sizes in which the viruses are found, and base them on data some of which may be found elsewhere in this review and some included in this section.

First we intend to review the data on which Elford's (1933) calibration is based:

(*a*) *Chromobacterium prodigiosum*. The short diameter of this organism was measured microscopically by Elford and found to be from 0·5 to 1 μ. There seems to be no reason for doubting this value.

(*b*) Bovine pleuropneumonia. The size of the smallest particles of this organism was measured by Barnard on his ultra-violet light microscope and found to be 0·2 μ. We do not comment on this value as we have no data on which to base any alternative value.

(*c*) Gold sols produced by reduction. The sizes were obtained by counting a known mass of particles. These sols are known to be polydisperse (Rinde, quoted by Svedberg, 1928). Furthermore, as the solutions were filtered in sodium oleate solutions, it is possible that the particles were solvated. Such an effect has been demonstrated by Svedberg & Rinde (1924) for gold sols stabilized by gelatin, and the protection by sodium oleate may have a similar effect. Such an effect, if present, would increase the size of the particles.

(*d*) Oxyhaemoglobin (horse) is taken as being 5 mμ in diameter from the ultracentrifuge values of Svedberg (1930) and 5·46 mμ in diameter from the diffusion results of Northrop & Anson (1928–9). The former value appears to have been deduced by Stokes's law from the sedimentation constant given by Svedberg, as no size is quoted by him. Furthermore, as the dissymmetry constant is given as 1·25, the molecule is obviously hydrated, enormously elongated or flattened (see § IX). The value given by Northrop & Anson is known to be incorrect (Anson & Northrop, 1936–7).

(*e*) Egg albumin is taken as being a sphere, 4·34 mμ in diameter (Svedberg, 1930). More recent data (Svedberg & Pedersen, 1940) suggest that it is either hydrated or aspherical. See also Adair (1939), and Adair & Adair (1936).

Further evidence in support of the calibration factors was given by Elford & Ferry (1936), the diameter of haemocyanin (*Helix pomatia*) being taken as 24·4 mμ from the incorrect data of Svedberg & Chirnoaga (1928). Edestin was also used as a standard, the

size being taken from data of Svedberg & Stamm (1929), since shown to be incorrect (Svedberg & Pedersen, 1940).

In view of the rather unsatisfactory state of these standards, we propose instead the following series:

(*a*) *Chromobacterium prodigiosum*, 500 mμ (small diameter).

(*b*) Vaccinia virus, 274 × 200 mμ (see § X).

(*c*) Tomato bushy stunt virus, 37 mμ (see § X).

(*d*) *Helix* haemocyanin, 31 mμ. This figure is based on the following data: $s_{20} = 98{\cdot}9 \times 10^{-13}$, $M_e = 6{,}600{,}000$ and $D_{20} = 1{\cdot}38 \times 10^{-7}$ (Svedberg & Pedersen, 1940). Further information is given in the section on the ultracentrifuge and diffusion. We are assuming the particle to be spherical and hydrated in solution. This assumption is substantiated by the investigations of von Ardenne (1940) with the electron microscope which show the anhydrous molecules to be spheres.

(*e*) Haemoglobin (horse). X-ray data indicate that the unhydrated horse haemoglobin molecule approximates in shape to a flat platelet about 3·6 mμ thick with an average diameter of 5·6 mμ: its greatest length is 6·4 mμ (Perutz, 1942, and private communication). Taking $s_{20} = 4{\cdot}4 \times 10^{-13}$ and $D_{20(\text{calc.})} = 6{\cdot}4 \times 10^{-7}$ into account, we propose a size of between 5 and 6·8 mμ for the largest diameter of the smallest cross-section of the molecule in solution. We do not regard Neurath's values (1939) for the size of haemoglobin as being correct. This view is strengthened by the fact that he regards most proteins, including *Helix* haemocyanin, as being greatly elongated. In his view horse haemoglobin is a prolate ellipsoid of $15{\cdot}5 \times 3{\cdot}2^2$ mμ. Thus we arrive at the following table (Table 1). It appears from this table that there is no systematic change in the ratio

Table 1. *Revised estimate of relationship between 'average pore diameter' and size of particle retained by gradocol membranes*

Particle	Size mμ	A.P.D. by filtration mμ	Size/A.P.D.	Reference
Chr. prodigiosum	500	750	0·67	Elford (1933)
Vaccinia	200	250	0·80	Elford & Andrewes (1932*a*)
Tomato bushy stunt virus	37	40	0·93	Smith & MacClement (1941)
Helix haemocyanin	31	55	0·56	Elford & Ferry (1936)
Haemoglobin	5–6·8	10	0·5–0·68	Elford (1933)

size/A.P.D. with size, and so we propose that filtration results obtained by the use of gradocol membranes should be calculated in all cases by the ratio, size of particle/A.P.D. = 0·55–0·95, the variation apparently being due to the experimental error inherent in the method. It will be noted that, although haemoglobin is said to dissociate into half-molecules on dilution, it is unlikely that the halves of an almost spherical particle will be able to pass through a smaller pore than the whole particle.

V. SEDIMENTATION (EXPERIMENTAL)

The determination of size or of sedimentation constant of viruses has been made by means of two classes of apparatus:

(*a*) Ultracentrifuges. Centrifuges specially designed to allow sedimentation to proceed unhindered by convection or vibration. These include the high-speed oil turbine centrifuges and the equilibrium centrifuges of Svedberg, and the air turbine-driven ultracentrifuges developed in America by Beams, Pickels, Wyckoff and others.

(*b*) Laboratory centrifuges, generally of high speed, adapted for the purpose of measuring sedimentation (Bechhold & Schlesinger, 1931 *a*, *b*; Schlesinger, 1936; McIntosh & Selbie, 1937; Elford, 1936).

The machines in the first class, the ultracentrifuges, are carefully constructed and balanced machines, running in a vacuum or in hydrogen, and specially thermostated to avoid changes in temperature. The cells in which sedimentation is carried out are sectorial in shape and bounded by optical windows of quartz to allow the sedimentation to be followed by optical methods.

In order to follow sedimentation optically, it is essential that the virus preparation under investigation should be as free from other high molecular weight substances as possible. In practice, this consideration is often overlooked and therefore it seems likely that some, at least, of the sedimentation constants recorded may not actually be due to the viruses themselves. Several high molecular weight substances have been observed in normal animal and plant tissues (Taylor, Sharp, Beard & Beard, 1941; Gard & Pedersen, 1941; Price & Wyckoff, 1938, 1939; Loring, Osborn & Wyckoff, 1938), and in view of the now extensively practised method of differential centrifugation it is as well to remember that such methods will only tend to isolate particles having similar size ranges. In fact, preparations made from suspensions of collodion particles in the manner usually adopted for preparing the elementary bodies of vaccinia virus have been investigated by Smadel, Pickels, Shedlovsky & Rivers (1940), and when examined on the ultracentrifuge have given sedimentation constants almost identical with the vaccinia virus preparations.

Two main optical methods of observation have been used to follow sedimentation: (*a*) light absorption, (*b*) refractive index methods. The former method generally employs light from a mercury arc lamp filtered through chlorine and bromine filters and consists of light largely of wave-lengths 265, 254 and 248 mμ. The property of absorbing these wave-lengths is common to most proteins and to many other substances, and so the method has no particular specificity. In addition, a small quantity of a heavier component may easily be masked by the more heavily absorbing material which predominates. The concentration range of the substance under investigation is limited by photographic considerations, a serious defect in cases when the sedimentation constant is not independent of concentration as in the case of tobacco mosaic virus (Lauffer, 1940).

The most generally used refractive index method is the scale method of Lamm & Polson (1936). While the property of having a different refractive index from water is not characteristic of virus solutions, this method is more sensitive to the presence of more than one component in the sample than the light absorption method.

Whenever the purity and homogeneity of the preparation is in doubt, it would seem to be desirable to use the separation cell of Tiselius, Pedersen & Svedberg (1937) to confirm the optical observations by virus activity measurements. This method has been used very successfully by Gard & Pedersen (1941) who isolated three substances with sedimentation constants of 40, 160 and 210×10^{-13} from brains of mice affected with mouse encephalomyelitis and demonstrated that the activity was associated with the middle component which was not present in normal mouse brains. Unfortunately, this seems to be the only virus preparation which has been investigated in this manner. As in diffusion measurements, sufficient salt concentration must be maintained to allow sedimentation to take place unhindered by charge effects. The absence of sufficient salt will result in a smaller value for the sedimentation constant and probably in a spurious sharpening of the boun-

dary. The values for tobacco mosaic virus given by Wyckoff (1937*a*) are probably too small for this reason. The sedimentation constant is calculated from the position of the boundary by means of the formula $s=\frac{dx/dt}{\omega^2 x}$, where x is the radius at the boundary at time t, and ω is the angular velocity in radians per second. Thus s represents the sedimentation velocity in cm./sec. in a unit field of force, and is usually expressed as corrected to water at 20° C. (s_{20}).

The second group of centrifuges consists of machines which have been improvised from existing equipment. The theory of sedimentation in such machines has either been calculated for the particular conditions of the experiment (Bechhold & Schlesinger, 1931 *a*, *b*) or else an attempt has been made to approximate to the conditions obtaining in an ultracentrifuge (Elford, 1936; Schlesinger, 1936). In general, sedimentation is followed by estimations of virus activity.

Bechhold & Schlesinger (1931 *a*, *b*) and Schlesinger (1934) have calculated the rate at which particles sediment from a completely stirred liquid. They consider a column of liquid stirred by convections and vibrations and having a small region at the bottom in which normal sedimentation takes place on to a surface retaining all particles reaching it. They deduce that the concentration of the supernatant liquid decreases at a rate expressed by the formula $C_t/C_0=e^{-(RK)t/h}$, where C_t is the concentration of virus in the supernatant liquid at time t, C_0 is the original concentration, R is the radius at the bottom of the tube, h is the height of column of liquid, and K is $\omega^2 s$.

It can be seen that, if all the conditions are fulfilled and centrifugation is carried out until C_t is very small, s can be determined relatively accurately even if C_t is only known to the nearest tenfold dilution.

In practice, tubes with conical or flat bottoms are used, with or without filter paper, or with kieselguhr or sintered glass to make the sediment adhere. It will be seen that the smallest quantity of sediment resuspended by stirring will cause the answer to be grossly incorrect when C_t becomes small, and yet the stirring must be adequate to prevent boundary formation which would invalidate the calculation. Bechhold & Schlesinger have given data which suggest that their conditions are fulfilled in their centrifuge.

The results are expressed, not in sedimentation constants but as radii of hypothetical spheres, the densities of which are either obtained by centrifuging in media of different densities or are assumed. Their results agree reasonably well with other values when formally expressed.

Elford (1936), in view of the discrepancy between his filtration figures and the values obtained by the Bechhold-Schlesinger technique, developed a new method using a high-speed laboratory centrifuge and spinning in inverted capillary tubes to prevent convection. Assuming that a boundary was formed, he developed a formula relating the quantity of the virus remaining in the capillary with the size of the virus, assuming it to be spherical, with known or assumed specific gravity. The formula given is

$$r=\sqrt{\frac{9\eta \log_e X}{2(\sigma-\sigma_0)\omega^2 t}}, \quad \text{where} \quad X=\frac{l+x_1}{l\frac{C_t}{C_0}+x_1},$$

where x_1 is the radius at the top of the capillary, l the length of the capillary, C_t is the mixed concentration of the virus in the capillary at the time t and $(\sigma-\sigma_0)$ is the specific

gravity difference between the solute and the solvent. We do not agree with McBain & Leyda's (1941) criticism of this formula. The formula applies to convectionless sedimentation in the capillary, but Elford gives data suggesting that it is not entirely convectionless, at any rate at the open end of the capillary. As a result, the value deduced for the radius of the particle, instead of being independent of C_t/C_0, diminishes as C_t/C_0 diminishes. The error, which is likely to increase the more C_t/C_0 departs from unity, is appreciable when $C_t/C_0 = 0{\cdot}5$ and serious for $C_t/C_0 = 0{\cdot}1$ or less. Elford suggests the procedure of calculating estimates of radius for different values of C_t/C_0 and extrapolating back to $C_t/C_0 = 1$, which should give an accurate result. His curves suggest that the estimate of size at $C_t/C_0 = 0{\cdot}1$ is about two-thirds of the extrapolated size.

However, in actual practice (Elford & Andrewes, 1936) runs are made with $C_t/C_0 = 0{\cdot}1$ without correction, presumably because it is difficult to estimate changes in concentration less than this by biological methods. The true values are therefore likely to be up to 50% higher than the values given. The agreement between individual runs on Elford's centrifuge is not very good.

Polson (1941) has described a capillary cell which would appear to eliminate some of the errors inherent in Elford's technique. McIntosh & Selbie (1937) describe a centrifuge of the air-driven spinning-top type which they use for the study of viruses. They do not state whether they believe the sedimentation to be convectionless or not, and from their data it would seem as if convection does occur, but in the case of the larger viruses is probably insufficient to maintain complete mixing as in the Bechhold & Schlesinger centrifuge. They use two formulae to arrive at the size of their particles:

(*a*) They plot log (C_t/C_0) against time, and assume that the gradient, tan θ, of the curve is proportional to the square of the diameter of the virus particle. Taking *Staph. aureus* as a standard, they are able to deduce the sizes of viruses spun in the same cell and at the same speed. This method is valid providing all the viruses are spherical and have the same density as *Staph. aureus*, and providing mixing in the cell is sufficient to keep the concentration in the supernatant uniform.

(*b*) They use a 'modified Stokes's law equation'. This equation is incorrect, but again assuming mixing in the cell to be adequate, the values deduced for the virus diameters should be proportional to the true values. We calculate that with their dimensions of cell, true values should be derivable from their incorrectly calculated values by increasing the latter by some 40%.

Schlesinger (1936) and Schlesinger & Galloway (1937) have described a method of obtaining sedimentation constants by means of a 'Sharples' supercentrifuge bowl lined with a weak agar gel. Using a very thin column of liquid, they assume that sedimentation is undisturbed by convection and that the particles reaching the agar are held sufficiently firmly to prevent their resuspension when the machine is stopped. Their data indicate that these conditions are not fulfilled, especially when C_t/C_0 becomes small. Irreversible volume changes in the gel were not observed under the experimental conditions. s is calculated in the usual way.

Sedimentation equilibrium

Sedimentation equilibrium measurements in the ultracentrifuge (Svedberg, 1926) are undoubtedly the soundest means, as judged from a purely theoretical standpoint, of finding the mass of particles. Unfortunately, the practical difficulties in applying the

technique to the study of viruses are enormous. In measurements of sedimentation equilibrium obtained in the orthodox Svedberg type of ultracentrifuge it is necessary to extend the run for days or even weeks to ensure that equilibrium has been attained in the case of viruses. During this time the centrifuge rotor must run at constant speed and constant temperature and without vibration. In consequence few measurements have been made by this method. Eriksson-Quensel & Svedberg (1936) investigated tobacco mosaic virus and runs of 17 and 23 days were made. The results were unsatisfactory on account of the polydispersity of the preparation, but gave general agreement with values calculated from sedimentation velocity experiments based on the assumption of a value for f/f_0 of 1·3, which is on the small side for this virus especially in an aggregated specimen.

Measurements have also been made by McFarlane & Kekwick (1938) of the sedimentation equilibrium of tomato bushy stunt virus. Their figures are discussed in § X.

Schlesinger (1936) and Schlesinger & Galloway (1937) have attempted to measure sedimentation equilibrium in viruses in a Sharples supercentrifuge. They use a thin layer of agar gel to line the cylinder and cover this with a virus suspension contained in an equal volume of liquid. As the total height of the column of solution is a fraction of a millimetre, equilibrium is attained very rapidly. At the conclusion of the experiment the supernatant liquid and the gel layer are examined biologically for virus activity. The assumption that viruses can sediment or diffuse normally through agar gels is made and some evidence is advanced in support of this. Further assumptions are that convections and vibrations are absent and that the equilibrium is not affected by the rather violent stopping of this type of machine. It may be pointed out in this connexion that the speed of the Sharples may drop from 10,000 r.p.m. to 0 in less than 30 sec.

VI. DIFFUSION (EXPERIMENTAL)

The diffusion constant of a substance (see § II) can be measured in a variety of ways, but, as the diffusion constants of the viruses are small, only a few experimental methods can be used, and comparatively few determinations have been made.

As in all methods involving the treatment of the particles as ideal molecules obeying the gas laws, it is essential to carry out measurements in salt solutions in order to eliminate charge effects, and several determinations have been made without this precaution having been taken (Hetler & Bronfenbrenner, 1930–1; Kalmanson & Bronfenbrenner, 1939–40; Hills & Vinson, 1938; Frampton, 1939*a*). In consequence, the value of such determinations is difficult to assess.

The methods used are as follows:

(*a*) *Free diffusion in a parallel column with refractometric measurement of the diffusion.* The apparatus of Lamm & Polson (1936) has been used in investigations in tobacco mosaic virus (Neurath & Saum, 1938; Frampton & Saum, 1939; Frampton, 1939*a*) and of the virus of tomato bushy stunt (Neurath & Cooper, 1940). As the progress of the diffusion is followed by a refractometric (scale) method, it is essential that the preparation should be free from other large molecules and substantially pure. The length of time required for a satisfactory experiment is rather large, and the danger of errors arising from convections and vibrations in the apparatus are correspondingly great. The interpretation of the results obtained may be effected in a variety of ways and the agreement between the answers obtained by these various methods may not be very good. For instance, Neurath & Cooper (1940) give the diffusion constant (uncorrected) for tomato bushy stunt virus as

$1{\cdot}36 \times 10^{-7}$ and $1{\cdot}18 \times 10^{-7}$ from the same data by two different formulae. The diffusion measurements on tobacco mosaic virus are difficult to interpret, as Fick's law is not obeyed (Frampton, 1939*a*) and the samples used were probably very inhomogeneous.

(*b*) *Free diffusion in a parallel column with analytical measurement.* Bourdillon (1941*a*) has described an apparatus in which the diffusion column may be sampled at a number of heights at the completion of the experiment, and has applied this method to the study of viruses (Bourdillon, 1941*b*). He notes that the experimental error of the method decreases as the boundary becomes more diffuse, but in measurements on the diffusion of viruses this condition is difficult to realize on account of the long time taken for the particles to diffuse.

The position of the boundary at the start of the experiment is not measured but is estimated at the end. While this procedure is sound from a theoretical point of view, the position of the boundary is actually in considerable doubt as it depends on the accuracy of the estimation of activity in the various layers of the diffusion column. Naturally the boundary is taken as the point in the column at which the concentration is 50% of the original.

The diffusion constant is estimated by plotting a graph of log of relative virus activity against height of column and determining the theoretical curve which fits the data best.

The procedure of diffusing from a strong salt solution into a dilute one or water, which Bourdillon uses to stabilize his column, is open to criticism and prevents any correction for viscosity of the solvent from being made. The results obtained from diffusion experiments on influenza A virus and mouse encephalomyelitis virus are explained on the assumption of the presence of more than one size of particle possessing virus activity.

(*c*) *Diffusion through a porous disk.* Northrop & Anson (1928–9) described a method for obtaining diffusion constants in a simple apparatus incorporating a sintered glass membrane, and this method has been used extensively on plant viruses (Waugh & Vinson, 1932; Hills & Vinson, 1938) and on bacteriophages (Hetler & Bronfenbrenner, 1930–1; Elford & Andrewes, 1932*b*; Northrop 1937–8; Kalmanson & Bronfenbrenner, 1939–40). The method has given very disappointing results on various proteins, largely on account of the theory of the apparatus not having been understood, and the majority of the virus experiments are also subject to some criticism, though it would appear that, with suitable precautions, the apparatus can give perfectly reliable measurements on the smaller viruses. There is some doubt as to whether the method can be applied to the greatly elongated plant viruses like tobacco mosaic virus, on account of the small size of the pores of the disk and the great length of the particles. In addition, the measurements of Hetler & Bronfenbrenner (1930–1), Kalmanson & Bronfenbrenner (1939–40), Waugh & Vinson (1932), and Hills & Vinson (1938) are based on an incorrect value for the diffusion constant of hydrochloric acid (Anson & Northrop, 1936–7) and were made in weak broth or distilled water and are therefore unreliable on account of charge effects. Recently we have investigated the method and have obtained consistent results (unpublished) for the diffusion constant of tomato bushy stunt virus and for carboxyhaemoglobin.

In our opinion, the method should prove to be very useful in the investigation of viruses, as the time required for a determination is much smaller than is necessary with the other methods. In addition the virus may be estimated by activity in an impure condition, and as the experimental error in the diffusion constant is directly proportional to the error in the estimation, the apparatus has some considerable advantages.

VII. SEDIMENTATION AND DIFFUSION (METHODS OF CALCULATION)

For a virus which is spherical and not hydrated in solution and of which the specific volume is known, sedimentation and diffusion experiments give independent estimates of the size. The agreement between these estimates is a test of the correctness of the presumption of spherical shape and no hydration. If the two estimates do not agree, it is not possible to distinguish between hydration and asphericity as the cause, unless some third datum bearing on shape or hydration is available.

Spherical viruses

The following considerations apply to spherical viruses which may be hydrated in solution. Exact spherical shape is not essential, since the modification introduced into the diffusion and sedimentation formulae is not serious for particles whose axial ratio does not exceed 2 : 1. The radius r for near spheres is of course the radius of the sphere of equal volume.

In evaluating formulae the following numerical values are adopted: $R=8{\cdot}314\times10^{7}$, $T=20°$ C. $=293{\cdot}2°$ absolute, $N=6{\cdot}023\times10^{23}$. For water at 20° C.: $\rho=0{\cdot}9982$ and $\eta=0{\cdot}010087$. M, the molecular weight, r, the radius of the particle in centimetres, and V, the specific volume, refer to the virus in the state of hydration existing in the solution; M_0, r_0 and V_0 to unhydrated virus. If hydration occurs to the extent of w g. of water combining with 1 g. of virus, then

$$\frac{V}{V_0}=\frac{1+w/V_0\rho}{1+w};\quad M=M_0(1+w);\quad r=r_0\left(1+\frac{w}{V_0\rho}\right)^{\frac{1}{3}}. \tag{1}$$

In addition to the above the fundamental formulae are:

$$s=\frac{M(1-V\rho)}{6\pi\eta Nr}\text{ for sedimentation,} \tag{2}$$

$$D=\frac{RT}{6\pi\eta Nr}\text{ for diffusion,} \tag{3}$$

$$MV=\tfrac{4}{3}\pi r^3N. \tag{4}$$

We do not list a separate formula for sedimentation equilibrium experiments, since these can be regarded as measuring not s or D separately but s/D, and are covered by case 3 below. Depending upon the amount of experimental data available, the following cases may arise:

Case 1. *Diffusion constant known but not sedimentation constant.*

In this case it is not possible to deduce whether or not the particle is hydrated. The diameter is immediately given from formula (3) and is

$$2r=\frac{RT}{3\pi\eta ND}=\frac{4{\cdot}258\times10^{-13}}{D_{20}}. \tag{5}$$

This refers to the diameter of the hydrated molecule, if hydration occurs. If V (the specific volume of the hydrated molecule) is known we may deduce the hydrated molecular weight from (4), viz.

$$M=\frac{R^3T^3}{162\pi^2\eta^3N^2}\frac{1}{VD^3}=\frac{2{\cdot}434\times10^{-14}}{VD_{20}^3}. \tag{6}$$

Case 2. *Sedimentation constant known but not diffusion constant.*

In this case also it is not possible to decide whether the particle is hydrated. V must be known to deduce the virus size from the sedimentation constant. From (2) and (4)

$$2r=\left(\frac{18\eta Vs}{1-V\rho}\right)^{\frac{1}{2}}=0{\cdot}4261\left(\frac{Vs_{20}}{1-V\rho}\right)^{\frac{1}{2}}. \tag{7}$$

$$M=9\pi N(2V)^{\frac{1}{2}}\left(\frac{\eta s}{1-V\rho}\right)^{\frac{3}{2}}=2{\cdot}440\times10^{22}V^{\frac{1}{2}}\left(\frac{s_{20}}{1-V\rho}\right)^{\frac{3}{2}}. \tag{8}$$

In the event of hydration, then the above formulae refer to the hydrated particle.

It may often happen, however, that the specific volume is known only for the unhydrated virus. If this value (V_0) is inserted in formulae (7) and (8) then the estimates obtained for the size will be even smaller than the size of the unhydrated molecule.

The estimate found for the molecular weight (M_a) will be related to the unhydrated molecular weight (M_0) by the formula

$$M_0=M_a\left(1+\frac{w}{V_0\rho}\right)^{\frac{3}{2}}, \tag{9}$$

while the apparent radius r_a will be related to the unhydrated radius r_0 by the formula

$$r_0=r_a\left(1+\frac{w}{V_0\rho}\right)^{\frac{1}{3}}. \tag{10}$$

Case 3. *Sedimentation and diffusion constants known.*

If both sedimentation and diffusion constants are known, as well as V_0, the partial specific volume of the (unhydrated) virus, then we are in a position to determine the degree of hydration.

s and D together will give us the molecular weight and diameter of the virus as it is in solution.

From (2), (3) and (4) we deduce

$$2r=\frac{RT}{3\pi\eta ND}=\frac{4{\cdot}258\times10^{-13}}{D_{20}},$$

$$M=\frac{RTs}{D}+\frac{R^3T^3\rho}{162\pi^2N^2\eta^3D^3}=2{\cdot}438\times10^{10}\frac{s_{20}}{D_{20}}+\frac{2{\cdot}429\times10^{-14}}{D_{20}^3}, \tag{11}$$

while the partial specific volume of the virus in the solution is

$$V=\left(\rho+\frac{162\eta^3N^2\pi^2D^2s}{R^2T^2}\right)^{-1}=(\rho+1{\cdot}002\times10^{24}D_{20}^2s_{20})^{-1}. \tag{12}$$

In the event of hydration $2r$, M and V refer to the hydrated virus.

If V_0, the specific volume of the unhydrated virus, is known, comparison with V given by equation (12) will enable us to decide whether there is any hydration which will show itself by V exceeding V_0. We may then deduce the degree of hydration, since

$$w=\frac{V-V_0}{1/\rho-V}, \tag{13}$$

or in terms of D, s and V_0

$$w=\frac{R^2T^2}{162\pi^2\eta^3N^2}\frac{\rho(1-V_0\rho)}{D^2s}-V_0\rho=0{\cdot}998\times10^{-24}\frac{\rho(1-V_0\rho)}{D_{20}^2s_{20}}-V_0\rho. \tag{14}$$

Knowing V_0 as well as s_{20} and D_{20}, the size of the unhydrated molecule may be determined even though hydration occurs, by the formulae

$$M_0=\frac{RTs}{D(1-V_0\rho)}=2{\cdot}438\times10^{10}\frac{s_{20}}{D_{20}(1-V_0\rho)}, \tag{15}$$

$$2r_0=\left\{\frac{6RTV_0s}{\pi N(1-V_0\rho)\,D}\right\}^{\frac{1}{3}}=4{\cdot}260\times10^{-5}\left\{\frac{s_{20}V_0}{D_{20}(1-V_0\rho)}\right\}^{\frac{1}{3}}. \tag{16}$$

VIII. PARTIAL SPECIFIC VOLUME AND HYDRATION

In order to obtain the size and weight of a virus from the data outlined in previous sections, it is necessary to know the partial specific volume, V. V is defined as the change in volume of a large quantity of solvent resulting from the addition of 1 g. of the solute, all other factors being kept constant. In general the specific volume of the solute at the concentrations used in the experiment is determined and this, if it does not vary with concentration, is equal to V. For convenience we will define the density ρ of a substance as $1/V$.

It will be realized that density defined in this way is mass divided by volume, as in the ordinary definition, but that 'volume' is the volume of solvent displaced when the substance is dispersed in the solvent, not the volume occupied by the dry material in bulk. Thus the reciprocal of the partial specific volume of unhydrated bushy stunt virus is 1·35, but the density of the dry crystals, in the sense of mass divided by the volume occupied by the crystals, is 1·12. The difference is presumably due to spaces between the virus particles in the crystals.

Density and specific volume are required in this work in two connexions: (*a*) in the term $1-V\rho$ in the centrifugation equation. Since this term is the Archimedian buoyancy correction, V means the volume of solvent displaced by a virus particle, divided by the weight of the virus particle; (*b*) in converting the sizes of a particle deduced from Stokes's law to the weight of the particle. Here again size means the space occupied by the virus particle in the fluid, and hence the density of the dry material in bulk is not the relevant density.

When viruses or proteins are dispersed in aqueous solution, a certain amount of water becomes attached in many, perhaps in all, cases. The effect of this water, moving with the particles as a kinetic unit, is to increase the frictional constant without increasing the centrifugal force, if the solvent is water or a dilute buffer solution. The partial specific volume of the hydrated particle is greater than that of the anhydrous solid in the ratio

$$V/V_0=\left(\frac{1+w/V_0\rho}{1+w}\right), \text{ as given by formula (1),}$$

where w is the weight in grams of the water combined with 1 g. of solute, it being assumed, in the absence of definite evidence to the contrary, that the volume occupied by the water in the hydrated particle is the same as when it is not combined.

It is evident, therefore, that the value of the specific volume needed is that of the hydrated particle, V, and not that of the anhydrous particle, which we will call V_0.

It is possible to conceive that water can combine with a particle in two ways:

(*a*) In mechanical combination. Water moves with the particle by virtue of its being held by friction, i.e. in a spongy, porous structure. It possesses all its usual properties and can also act as a solvent. Hence the composition of this water is identical with that of the solvent. By 'solvent' we understand the water in which the particle is suspended, plus any additional substance which has been added and which alters the apparent viscosity and density, etc., of the solution, e.g. sucrose or salt.

(*b*) In chemical combination. The water is attached to polar groups on the surface and possibly inside the particle. Its solvent properties may be altered to such an extent that it is unable to act as a solvent for the other constituents of the bulk of the solvent.

The fact that there are these two possibilities complicates the determination of V. V_0 of course is easily determined by finding the mass of dry solid contained in a volume of solution of known density. Most indirect methods of determining V depend upon the assumption that the water of hydration does not possess normal solvent properties. If a virus is immersed in a solution of density ρ, such that $(1-V\rho)=0$, then, no matter how intense a centrifugal field is employed, no sedimentation will take place. This principle was employed by MacCallum & Oppenheimer (1922) to determine V for vaccinia, and has been used since by many workers (Bechhold & Schlesinger, 1931 *a*, *b*; Schlesinger & Galloway, 1937; Elford, 1936; McIntosh & Selbie, 1937, etc.). Besides assuming that the water of hydration is incapable of dissolving other constituents of the solvents, which is certainly untrue if heavy water is used to increase the density of the solvent (Svedberg & Eriksson-Quensel, 1936), the solvent itself must not injure the virus particles or increase or decrease their volume. Furthermore, there must be no adsorption of constituents of the solvent in the particles.

Bechhold & Schlesinger (1933*a*) noticed that in strong sucrose solutions the virus they were employing apparently increased in density, and they suggested the possibility of determining V by spinning viruses in solutions of varying density and, by inserting the values of sedimentation rates obtained into their usual equations, solving V. Similar suggestions have been made by Elford (1936), Wyckoff (1937–8*a*) and Pickels & Smadel (1938). A typical equation is that of Wyckoff:

$$\frac{s_{20w}}{s_{20s}}=\frac{(1-V\rho)_w}{(1-V\rho)_s},$$

where the suffixes w and s denote that the experiment was carried out in solvents of different composition; s_{20} is corrected for viscosity but not for density.

It will be seen that if s_{20} is plotted against ρ, provided that no change in the extent of hydration takes place, s_{20} will become zero when $V=1/\rho$. In the event of the water being attached by purely mechanical forces s_{20} will become zero when $V_0=1/\rho$.

In actual practice it is often found that the curve of s_{20} against ρ is not linear (Smadel, Pickels & Shedlovsky, 1938) and that in the case of vaccinia virus in sucrose solution the value of V varies from 0·862 in very dilute solution to a value 0·8, approximating to $V_0=0{\cdot}794$ (McFarlane, Macfarlane, Amies & Eagles, 1939) in stronger solutions, and it would seem likely that shrinkage of the whole virus particle takes place. In glycerol and urea solutions, however, a linear curve of s_{20} against ρ is given, extrapolating to a value of V corresponding to V_0. It would appear from this that the water of hydration of vaccinia virus is a free solvent for both urea and glycerol or that these substances combine

with the virus. The virus is inactivated by urea solution but not by glycerol or sucrose. If we assume that vaccinia virus shrinks on drying and therefore that the dry particles have a specific volume $V_0 = 0{\cdot}794$, it is possible to calculate V on the basis of the electron microscope and centrifuge data. The electron microscope gives $2r_0 = 200\ \mathrm{m}\mu$, and as $V_0 = 0{\cdot}794$, hence from equation (16)

$$\frac{s_{20}}{D_{20}} = \left(\frac{1 - V_0\rho}{V_0}\right)\left(\frac{200 \times 10^{-7}}{4{\cdot}26 \times 10^{-5}}\right)^3 = 2{\cdot}703 \times 10^{-2},$$

and, as $s_{20} = 4910 \times 10^{-13}$ (Pickels & Smadel, 1938), therefore $D_{20} = 0{\cdot}182 \times 10^{-7}$. Again using formula (12) we deduce $V = 0{\cdot}862$.

It is also possible to obtain an estimate of the water of hydration by the partition of a solute between water and the virus solution, assuming that the water of hydration is incapable of acting as a solvent for the substance in question, generally sucrose or salt. As with the centrifuge method, which is merely another way of determining the same thing, the apparent water of hydration decreases with increase of concentration of solute in the external medium. If measurements are made in very dilute solutions the difficulty of estimating small changes in concentration becomes large and therefore the results obtained are not very accurate. McFarlane *et al.* (1939) and Moriyama (1939) have determined the hydration of vaccinia virus by this method, and at low concentrations of the solute (sucrose or egg albumin) find that the apparent volume of water associated with the particles is some 7–8 times the volume of the dry virus. The former authors explain this improbable value by assuming that the water is present as an 'ionic atmosphere' which, though incapable of acting as a solvent, does not move with the particles when they sediment in the centrifuge. Smadel *et al.* (1938), however, do not think it plausible to postulate a layer of water, many molecules thick on the surface of the virus.

It is possible to obtain the value of V for several spherical particles from purely theoretical reasoning. Vaccinia virus, mentioned above, has $V = 0{\cdot}862$, bushy stunt virus has $V = 0{\cdot}853$, if the values $D_{20} = 1{\cdot}15 \times 10^{-7}$ and $s_{20} = 131{\cdot}5 \times 10^{-13}$ are accepted. *Helix* haemocyanin having $D_{20} = 1{\cdot}38 \times 10^{-7}$ and $s_{20} = 98{\cdot}9 \times 10^{-13}$, gives

$$V = (\rho + 1{\cdot}002 \times 10^{24}\, D_{20}^2\, s_{20})^{-1} = (0{\cdot}9982 + 0{\cdot}1887)^{-1} = (1{\cdot}1869)^{-1},$$

therefore $V = 0{\cdot}843$ (see equation (12)).

It is of interest to note that these values are of the same order as those obtained for viruses by centrifuging so we include a table of values (Table 2) of V and V_0 of some proteins and viruses obtained by the various methods discussed above.

We conclude that there is good evidence for supposing that viruses and proteins are hydrated in solution.

It can be seen that the values for V_0 of proteins and viruses are roughly the same. The exceptionally low values for alfalfa mosaic virus (Ross, 1941) and tobacco ringspot virus (Stanley, 1939) are hard to explain, but, in the former case, the contents of the pycnometer were not weighed but were estimated from a Kjeldahl nitrogen determination (Lauffer & Ross, 1940) which might have given a low value, and thus a low value of V_0. It seems that the higher value of V_0 for vaccinia virus is due to the presence of a fatty constituent. When defatted $V_0 = 0{\cdot}745$ (McFarlane *et al.* 1939).

It also seems probable that in weak buffer solution most viruses have a V of about 0·85, and it is possible that proteins may behave similarly. The implications of this in regard to the size of the virus have been dealt with in § VII.

Attempts have also been made to measure the volume of virus when centrifuged to the bottom of a tube (McFarlane *et al.* 1939; Moriyama, 1939), but it seems likely that the centrifugal fields used were insufficient to pack the sediment tightly against a sedimentation equilibrium. In the event of the virus particles not being spherical, it is not possible to measure the volume occupied by virus in the pellet, unless the shape of the particles and the mode of packing are known. In the case of uniform spheres, close-packing will probably take place and the volume occupied by the virus particles may be taken as about 74% of the total volume of the pellet.

Table 2. *The specific volumes of proteins and viruses as obtained by the methods outlined in* § VIII

	$V_{calc.}$	V weak salt or sugar	V strong salt or sugar	V_0		$V_{calc.}$	V weak salt or sugar	V strong salt or sugar	V_0
	Proteins					Animal viruses			
Ovalbumin	—	—	—	0·742 (1)	Foot and mouth	—	—	0·715 (13)	—
Haemoglobin	—	—	—	0·746 (1)	Herpes	—	—	0·87 (3)	—
Edestin	—	—	—	0·744 (17)	Influenza A	—	—	0·834 (6)	—
Haemocyanin	*0·843*	—	—	0·738 (16)	Rabbit fibroma	—	—	<0·8 (12)	—
					Rabbit papilloma	—	—	<0·8 (12)	0·756 (9)
	Plant viruses				Rous sarcoma	—	—	<0·87 (6)	—
Alfalfa mosaic	—	—	—	0·675 (10)	Vaccinia	*0·862*	0·862 (14)	0·80 (14)	0·794 (8)
Tobacco mosaic	—	—	—	0·73 (2)					
Tobacco ringspot	—	—	—	0·636 (15)		Bacteriophages			
Tomato aucuba mosaic	—	—	—	0·74 (2)	'Bacteriophage' Coli W.L.L.	—	—	0·875 (11)	—
Tomato bushy stunt	*0·853*	—	—	0·739 (7)		—	0·82 (5)	—	—
					Staphylococcus K	—	0·82 (5)	0·80 (4)	—
					Staphylococcus (Northrop)	—	0·84 (18)	—	—

(1) Adair & Adair (1936).
(2) Bawden & Pirie (1937).
(3) Bechhold & Schlesinger (1933*a*).
(4) Elford (1936).
(5) Elford (1938).
(6) Elford & Andrewes (1936).
(7) McFarlane & Kekwick (1938).
(8) McFarlane *et al.* (1939).
(9) Neurath *et al.* (1941).
(10) Ross (1941).
(11) Schlesinger (1933*a*).
(12) Schlesinger & Andrewes (1937).
(13) Schlesinger & Galloway (1937).
(14) Smadel *et al.* (1938).
(15) Stanley (1939).
(16) Svedberg & Chirnoaga (1928).
(17) Svedberg & Stamm (1929).
(18) Wyckoff (1937–8*a*).

IX. The frictional ratio f/f_0 for non-spherical viruses

Most of the formulae (cp. § VII) used for deriving estimates of virus size from sedimentation and diffusion data depend upon the use of Stokes's formula for the friction experienced by a sphere of radius r moving at velocity v through a fluid of viscosity η, viz. $6\pi\eta vr$. If the virus is not markedly aspherical, having a ratio of maximum and minimum diameters not exceeding 2 for example, the use of Stokes's formula will give a value of r which to a close approximation will be the radius of the sphere of volume equal to that of the virus. Some viruses, however, are rod shaped with a length more than ten times their breadth, and for these a better approximation is to consider them as prolate ellipsoids of revolution. Perrin (1934, 1936) has worked out a formula for the friction experienced by particles of this shape, supposed at random orientation as a result of Brownian movement. The friction experienced by a particle whose ratio of length to breadth is L/d may be expressed as $6\pi\eta vr\,(f/f_0)$, where f/f_0 is the frictional ratio given by Perrin as a function of L/d and conveniently tabulated by Svedberg & Pedersen (1940) whose table we embody in Table 3. r is here the radius of a sphere having a volume equal to that of the virus particle in the solution. It is evident that if we know L/d and can so deduce f/f_0, all the formulae of § VII may still be used with the simple

modification that wherever η occurs we replace it by $\eta\,(f/f_0)$. Thus in place of formula (7) we should have

$$2r = \left(\frac{18\eta\,\frac{f}{f_0}\,Vs}{1-V\rho}\right)^{\frac{1}{3}} = 0{\cdot}4261\left(\frac{f}{f_0}\right)^{\frac{1}{3}}\left(\frac{Vs_{20}}{1-V\rho}\right)^{\frac{1}{3}}.$$

Formulae which do not involve η need no modification, and we can thus see at a glance that formulae (15) and (16) give correct estimates of the size of the virus even though its shape is unknown.

In some treatments of this subject the quantity f/f_0 is made to include besides the increased friction experienced by a particle which is rod shaped instead of spherical also the increased friction experienced by a particle which increases its size by hydration. In our treatment however, since the effect of hydration is explicitly allowed for, f/f_0 differs from unity only on account of asphericity, and is unity for spherical viruses whether hydrated or not.

From sedimentation and diffusion data alone, with no prior knowledge of either shape or hydration, it is impossible to tell the degree of hydration and the extent of the departure from the spherical shape except when both are zero. It is therefore customary to use some independent method to determine the shape, e.g. electron microscopy, X-ray crystallography, anisotropy of flow, or viscosity. The electron microscope will of course give the length and breadth of the unhydrated particle. Since hydration of a rod-shaped particle is likely to increase its width in greater proportion than its length, L/d so determined will not be identical with L/d for the particle in solution, in the event of hydration occurring, and this must be allowed for. For the method of calculation in such cases the reader is referred to § X, where a detailed calculation for tobacco mosaic virus is made.

A method which has frequently been employed for the determination of the axial ratio L/d is the measurement of the specific increment of viscosity. When a solute is dissolved in a solvent of viscosity η_0 the viscosity η of the solution is increased above that of the solvent according to the formula

$$\eta/\eta_0 - 1 = G\{2{\cdot}5 + \tfrac{1}{16}(L/d)^2\}. \tag{17}$$

This formula, due to Kuhn and discussed by Svedberg & Pedersen (1940), Lauffer (1938) and Robinson (1939 *a*, *b*) treats the virus particles as long cylinders maintained by Brownian movement at random orientation. For this formula to be valid it is necessary for small rates of shear to be used to avoid alignment of the particles, and for the virus concentration to be low. G is the volume of virus dissolved in unit volume of solution. It might be thought that in the event of hydration occurring this formula would lead to the axial ratio of the hydrated virus. Actually it does not, but leads approximately to the axial ratio of the unhydrated virus. This is because G, the volume of hydrated virus per unit volume of solution, is in practice not known, and instead G_0, the volume of unhydrated virus per unit volume of solution, is substituted in the formula. We have then

$$G = G_0(1 + w/V_0\rho) \quad \text{and} \quad d = d_0(1 + w/V_0\rho)^{\frac{1}{2}}, \tag{18}$$

where ρ is the density of the solvent, V_0 the specific volume of the unhydrated particle and w the weight of water in grams per gram of virus, d and d_0 being the widths of the hydrated and unhydrated viruses respectively, and hydration being supposed to increase the *width* but not in the same proportion the *length* of a rod shaped-virus. Thus

$$\eta/\eta_0 - 1 = G_0\{2{\cdot}5(1 + w/V_0\rho) + \tfrac{1}{16}(L_0/d_0)^2\}. \tag{19}$$

Since the first term on the right-hand side is, for a highly elongated virus, much smaller than the second, exact knowledge of w is not required to calculate L_0/d_0 from the observed viscosity increment, and in practice w is neglected completely.

In Table 3 the first column gives L/d, the axial ratio for a rod-shaped virus, the second column f/f_0 according to Perrin's formula, and the third column

$$10^{-3}\{2{\cdot}5+\tfrac{1}{16}(L/d)^2\},$$

i.e. the viscosity increment for a virus concentration of 1 c.c. of dry virus per litre of solution.

If no account is being taken of hydration, columns II and III of this table may be used for reading off directly the value of f/f_0 corresponding to any given viscosity increment. If, however, hydration to the extent of w g. of water per gram of virus occurs, the following procedure should be used. The axial ratio corresponding to the experimental viscosity increment is read off from column I, multiplied by $(1+w/V_0\rho)^{-\frac{1}{3}}$ to obtain the value for the hydrated virus, and the figure for f/f_0 which corresponds to this new axial ratio read off from column II.

Table 3. *Relation between axial ratio, frictional constant, and specific viscosity increment*

I Axial ratio	II f/f_0	III Viscosity increment	I Axial ratio	II f/f_0	III Viscosity increment
1·0	1·000	0·0025	12	1·645	0·012
1·2	1·003	—	14	1·729	0·015
1·4	1·010	—	16	1·829	0·018
1·6	1·020	—	20	1·996	0·028
1·8	1·030	—	25	2·183	0·042
2·0	1·044	0·003	30	2·356	0·059
3	1·112	0·003	35	2·518	0·079
4	1·182	0·004	40	2·668	0·102
5	1·255	0·004	50	2·946	0·159
6	1·314	0·005	60	3·201	0·228
7	1·375	0·006	70	3·438	0·309
8	1·433	0·007	80	3·658	0·402
9	1·490	0·008	90	3·867	0·508
10	1·543	0·009	100	4·067	0·628

B. THE SIZES OF VIRUSES

In Charts 1, 2 and 3 we have summarized published data on the sizes of animal viruses, plant viruses and bacteriophages.

X. The sizes of vaccinia, tomato bushy stunt, and tobacco mosaic viruses

In this section of the paper we discuss a limited number of viruses, information about which is available by a number of different methods, enabling an estimate of size to be given with some confidence. Calculations involving hydration, sedimentation, and diffusion are based on the formulae of §§ VII and IX.

Vaccinia virus

This virus is known to be hydrated in aqueous solution since the dry density is 1·26 (McFarlane *et al.* 1939) and the density in dilute buffer is 1·16 (Smadel *et al.* 1938) rising to 1·25 in 53% sugar solution. The change of density from 1·26 to 1·16 corresponds to 0·490 g. of water combining with each gram of dry virus (formula (13)).

Pickels & Smadel (1938) find a sedimentation constant of 4910×10^{-13}, from which, using the density 1·16 for the hydrated particle, we deduce the diameter of the particle

in solution to be 235·1 mμ. The dry particle diameter deduced from this size for the hydrated virus and the figure $w = 0{\cdot}490$ for the degree of hydration should be 200·2 mμ (formula (1)).

The size of the unhydrated virus can be measured on the electron microscope. Dr M. H. Salaman has kindly shown us some unpublished electron micrographs obtained at about 10,000 diameters magnification from a preparation submitted by him to Mr G. D. Preston of the Metallurgy Department of the National Physical Laboratory. On these photographs we measured the length and breadth of fifteen particles. Six of the particles were clearly bigger than the rest, and were probably clumped. The remaining nine were closely grouped about an average length and breadth of $234{\cdot}4 \times 170{\cdot}7$ mμ, the geometric mean of which is 200 mμ. The agreement with the indirectly calculated size is closer than we have a right to expect, in view of the fact that the magnification is subject to an uncertainty of 10%. It is evident that these determinations are mutually consistent and indicate a diameter for the hydrated particle of 235 mμ, and of the unhydrated particle of 200 mμ. These diameters are of course effective diameters, since the virus is not accurately spherical. The axial ratio revealed by the electron microscope (1·37 for the unhydrated particle) is not sufficient to invalidate Stokes's law.

Tomato bushy stunt virus

Electron microscope photographs of this virus, while not yet of a sufficiently high resolution to give an accurate estimate of size, show that the virus is nearly spherical. This is confirmed by the crystallographic classification of the crystals.

The most precise estimate of size at present comes from the X-ray diffraction studies of Bernal, Fankuchen & Riley (1938) on the crystalline form of the virus prepared by Bawden & Pirie. From the wet crystals they found reflexions which were consistent with the hypothesis of a body-centred cubical lattice, the cube edge being 39·4 mμ. This means that the virus particles in the crystal form a three-dimensional cubical pattern, each corner, and also the centre, of each cube being occupied by a single virus particle, or possibly by an identical group of particles.

Taking the former alternative it follows that a cube of edge 39·4 mμ of the crystal contains two virus particles plus water of crystallization. The density of the wet crystals being 1·286 it follows that the molecular weight of one particle plus associated water is $23{\cdot}7 \times 10^6$.

If the crystals are dried over P_2O_5 they shrink, and only a single reflexion is obtained. If it is assumed that the lattice is still of the body-centred type, and that the one reflexion obtained corresponds to the strongest of the nine reflexions obtained from the wet crystal, the angle of this reflexion indicates that the lattice edge is now 31·6 mμ instead of 39·4 mμ. This interpretation of the reflexion is strengthened by the fact that the crystal shows the same external degree of shrinkage.

The density of the dry crystals cannot be determined directly, but is calculated from the consideration that when the wet crystals shrink, their volume diminishes to a fraction $(31{\cdot}6/39{\cdot}4)^3$ of the wet volume and their weight diminishes to 45% of the wet weight (Bawden & Pirie, quoted by Bernal & Fankuchen, 1941*b*); this density is 1·120. Hence the molecular weight of the dry virus is $1{\cdot}12 \times (3{\cdot}16 \times 10^{-6})^3 \times 6{\cdot}023 \times 10^{23}/2 = 10{\cdot}65 \times 10^6$.

The partial specific volume of the dry virus being 0·739 (McFarlane & Kekwick, 1938) this molecular weight corresponds to a sphere of diameter 29·2 mμ for the unhydrated

Chart 1. *Animal Viruses*

Virus	Visible light microscope mμ	Ultra-violet light microscope mμ	Electron microscope mμ	Ultrafiltration mμ	Centrifugation 2r mμ	Centrifugation $s_{20} \times 10^{13}$	Viscosity mμ	Diffusion mμ	X-ray crystallography mμ	Irradiation mμ
Blue tongue	—	—	—	100–132 [55]	87–105 [55]	—	—	—	—	—
Borna disease	—	110–140 [25]	—	85–125 [25]	—	—	—	—	—	—
Canary virus	—	—	—	—	120 [13]	—	—	—	—	—
Chicken coryza	—	—	—	<135 [37]	—	—	—	—	—	—
Chicken laryngotracheitis	—	—	—	< 82 [37]	—	—	—	—	—	—
Dengue fever	250–400 [19]	—	—	—	—	—	—	—	—	—
Equine encephalomyelitis	—	—	—	20–30 [4]	32–39 [62]	245 [67]	—	—	—	—
				25–35 [62]	40 [67]	273 [60]				
Foot-and-mouth disease	150–300 [20]	40 [1]	—	7–16 [38]	14–20 [58]	40 [58]	—	—	—	—
				8–12 [22]	16–23 [58]	90 [58]				
					20 [58]					
					20 [37]					
Fowl plague	—	—	—	60–90 [31]	120–130 [10, 11]	—	—	—	—	—
				100–150 [43]						
Herpes	—	—	—	100–150 [30]	180–220 [12]	—	—	—	—	—
Horse sickness	—	—	—	—	46 [55]	—	—	—	—	—
Infectious ectromelia	—	130–140 [2]	—	100–150 [2]	—	—	—	—	—	—
Infectious myxomatosis	200–400 [56]	—	—	—	—	—	—	—	—	—
	310–360 [56]									
Influenza A	—	—	<15 [17]	80–120 [22]	87–99 [22]	—	—	12 + 400 [14]	—	—
Swine influenza				80–120 [24]						
Japanese epidemic encephalitis	—	—	—	20–30 [68]	—	—	—	—	—	—
Japanese summer encephalitis	250 [63]	—	—	—	—	—	—	—	—	—
Louping-ill	—	—	—	15–20 [26]	22–27 [62]	—	—	—	—	—
Lymphocytic choriomeningitis	—	—	—	40–60 [59]	52 [59]	—	—	—	—	—
					37·5 [59]					
					47 [59]					
					55 [59]					
Lymphogranuloma inguinale	300 [49]	—	—	150–200 [50]	—	—	—	—	—	—
				125–175 [16]						
Measles	250 [64]	—	—	—	—	—	—	—	—	—
Mouse encephalomyelitis	—	—	—	9–13 [66]	640 × 14 [36]	160–170 [36]	—	30 [14]	—	—
					590 × 19 [36]			$D_{20} = 0{\cdot}27\text{–}0{\cdot}33 \times 10^{-7}$ [36]		
Poliomyelitis	150–300 [20]	—	—	8–12 [23]	—	—	—	—	—	—
				12–17 [65]						
				ca. 15 [42]						
				50–110 [18]						
Pseudo-rabies	—	—	—	100–150 [35]	—	—	—	—	—	—
Psittacosis	280–380 [40]	—	—	200–300 [40]	—	—	—	—	—	—
Rabbit fibroma	—	—	—	125–175 [57]	126–141 [57]	—	—	—	—	—
Rabbit papilloma	—	—	—	23–35 [57]	40 [9]	250 [9]	—	$D_{25} = 0{\cdot}665 \times 10^{-7}$ [52]	—	—
					32–50 [57]	266·1–287·6 [52]				
Rabies	—	—	—	160–240 (street) [44]	—	—	—	—	—	—
				140–210 (fixed) [44]						
				100–150 (fixed) [24]						

Rabies	—	—	—	160–240 (street)[44] 140–210 (fixed)[44] 100–150 (fixed)[34] 100–150 (fixed)[60]	—	—	—	—	—	—
Rift valley fever	—	—	—	23–35[15] 20–30[15]	—	—	—	—	—	—
Rous sarcoma	—	—	—	100[23] <100[70] 70–100[22]	70[61] 60–70[22] 76[68]	532[61]	—	—	—	—
Sandfly fever	750–1300[19]	—	—	—	—	—	—	—	—	—
Sheep pox	—	—	—	170–260[41]	—	—	—	—	—	—
St Louis encephalitis	—	—	—	20–30[20] 20–30[68] 23–33[5]	—	—	—	—	—	—
Vaccinia	450[20]	—	150[39] 250[39]	125–175[21] 200[10, 11] 500[51]	236[54] 170–180[22] 103–240[48] 200[47] 190[46] 210–230[10, 11]	5000[46, 47] 5400[8] 4910[54]	—	—	—	—
Vesicular stomatitis	—	80–100[1]	—	70–100[3] 69–90[45] 70–100[33]	60[27]	—	—	—	—	—
Yellow fever	100–350[20] 350–450[19]	—	—	17–28[6] 17–28[7] 17–28[32]	12–19[53]	18–30[53]	—	—	—	—

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Chart 2. *Plant Viruses*

Virus	Visible-light microscope mμ	Ultra-violet light microscope mμ	Electron microscope mμ	Ultra-filtration mμ	Centrifugation $2r$ mμ	Centrifugation $s_{20} \times 10^{13}$	Viscosity mμ	Diffusion mμ	X-ray crystallography mμ	Irradiation mμ
Alfalfa mosaic virus	—	—	—	—	16·5 (22)	74 (22)	—	—	—	—
Aucuba mosaic virus	—	—	—	—	—	185, 220 (30)	—	—	—	—
Cucumber mosaic virus 1	—	—	—	15 (28)	—	—	—	—	—	—
Cucumber mosaic viruses 3 and 4	—	—	300 (length) (26) 275 × 15 (8)	—	—	173, 183 (8)	—	—	14·6 (dry) (2)	—
Potato virus *X*	—	—	—	15 (28) 33–50 (24)	9·8 × 433 (13)	113, 131 (14) 110 (27)	—	—	—	—
Tobacco mosaic virus	—	—	10–15 (diameter) (7) 300–1800 (length) (7) 15 × 280 (26)	11–15 (28) 13–20 (24) 15 (15)	50 (1)	193 + 226 (10) 174 + 200 (29) 93·4–244 (4)	12·3 × 430 (9) 11·5 × 725 (9) 14 × 720 (19)	8·18 (6) $D_{25} = 3 \times 10^{-8}$ (19) $D_{20} = 4{\cdot}6 \times 10^{-7}$ (in urea) (5) $D_{20} = 4{\cdot}5 \times 10^{-9}$ (5) $D_{22} = 0{\cdot}008$ cm.²/day (6)	17·3 (wet) (2) 15·9 (dry) (2)	—
Tobacco necrosis virus	—	—	20 (26)	13–20 (23)	—	130 + 58 + 220 (20) 112 (21)	—	—	—	19 and 14 (12)
Tobacco ringspot virus	—	—	—	16–21 (25) 15 (28) 13–20 (24)	19 (26)	115 (27)	—	—	—	—
Tomato bushy stunt virus	—	—	26 (26)	13–20 (24)	27·5 (16)	146 (16) 131·6 (10) 132 (11)	—	$D_{20} = 1{\cdot}15 \times 10^{-7}$ (16) $D_{20} = 1{\cdot}15 \times 10^{-7}$ (17)	27·6 (dry) (2) 34 (wet) (3)	—

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Chart 3. *Bacteriophages*

Virus	Visible-light microscope mμ	Ultra-violet light microscope mμ	Electron microscope mμ	Ultra-filtration mμ	Centrifugation $2r$ mμ	Centrifugation $s_{20} \times 10^{13}$	Viscosity mμ	Diffusion mμ	X-ray crystallography mμ	Irradiation mμ
Coli-dysentery phage	—	—	100 (length) (1) 20 (1)	15–20 (1)	—	—	—	—	—	—
Coli phage	—	—	100 (length) (1) 20 (1)	15–20 (1)	—	—	—	—	—	—
Coli phage T1	—	—	100 (length) (1) 20 (1)	15–20 (1)	—	—	—	—	—	—
Coli phage T2	—	—	100 (length) (1) 20 (1)	15–20 (1)	—	—	—	—	—	—
Coli phage T3	—	—	100 (length) (1) 20 (1)	15–20 (1)	—	—	—	—	—	—
Coli phage

Chart 3. *Bacteriophages*

Bacteriophage	Visible-light microscope mμ	Ultra-violet light microscope mμ	Electron microscope mμ	Ultra-filtration mμ	Centrifugation: 2r mμ	Centrifugation: $s_{20} \times 10^{13}$	Viscosity mμ	Diffusion mμ	X-ray crystallography mμ	Irradiation mμ
S 13	—	—	—	8–12 [5]	15–17 [3] 20 [16] 32 [12]	50 [18]	—	—	—	—
B. coli	25 [13]	—	40–80 [15]	—	45 [13]	—	—	—	—	—
D_{54}	—	—	—	30–45 [5]	—	—	—	—	—	—
S_{41}	—	—	—	30–45 [5]	—	—	—	—	—	—
B. megatherium	—	—	—	3–4 [10] 30–45 [20]	30–37 [19]	—	—	—	—	—
B. subtilis	—	—	—	8–12 [10]	—	—	—	—	—	—
Staphylococcus 'phage	60 [2]	—	—	—	—	—	—	—	—	—
B. coli S	—	—	—	2–5 [10]	—	—	—	—	—	—
Staphylococcus Krüger	—	—	—	6–9 [10]	—	—	—	—	—	—
B. coli B.W.	—	—	—	—	—	—	—	8·8 [7]	—	—
Staphylococcus (Northrop)	—	—	—	—	—	550–670 [21]	—	D = 0·001 cm.²/day [14]	—	—
Typhoid 'phage T_1	—	—	—	27–40 [22]	—	—	—	—	—	—
Typhoid 'phage T_{111}	—	—	—	20–30 [22]	—	—	—	—	—	—
Dysentery C 16	—	—	—	3–8 [10]	—	—	—	—	—	—
Dysentery YR 6	—	—	—	3–8 [10]	—	—	—	—	—	—
Dysentery Shiga	—	—	—	—	45 [12]	—	—	—	—	—
Dysentery DKA (Hospital)	—	—	—	69–90 [22]	—	—	—	—	—	—
Dysentery DKA (University)	—	—	—	27–40 [22]	—	—	—	—	—	—
Dysentery D (Nakamura)	—	—	—	27–40 [22]	—	—	—	—	—	—
Dysentery D (Flexner)	—	—	—	30–46 [22]	—	—	—	—	—	—
D_{20}	—	—	—	20–30 [5]	50 [16]	—	—	—	—	—
D_{16}, D_{48}, C_{36}	—	—	—	20–30 [5]	—	—	—	—	—	—
Staphylococcus B	—	—	—	—	—	—	—	—	—	26 [6]
Staphylococcus C	—	—	—	—	—	—	—	—	—	33 [6]
Staphylococcus D	—	—	—	—	—	—	—	—	—	43 [6]
C_{16}	—	*ca.* 50 [1]	—	—	79–90 [17]	—	—	—	—	50 [11]
'Phage 16	—	—	—	—	—	—	—	—	—	50–56 X-rays [9] 85 α rays [9]
C_{13}	—	—	—	15–20 [5]	—	—	—	—	—	32 [11]
P_{25}	—	—	—	—	—	—	—	—	—	36 [11]
Phytomonas pruni	—	—	—	11 [20]	—	—	—	—	—	—
Staphylococcus K	—	—	—	50–75 [5]	53–55 [12] 60–70 [3]	—	—	4·8 [8] 14 [8]	—	48 [11]
D_4 and D_{12}	—	—	—	50–75 [5]	—	—	—	—	—	—
B. coli P.C.	—	—	—	—	—	—	—	16–18 [8] 2·6–3·8 [8]	—	—

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virus. We can also derive an estimate for the diameter from the consideration that if the virus particles are in contact along the cube diagonal in the lattice, their diameter in that direction must be 27·4 mμ, and this therefore will be their diameter if they are exactly spherical. But as there is space available in the lattice for a diameter of 31·6 mμ parallel to the cube edges, the estimate 29·2 mμ for the mean diameter deduced from the molecular weight and density is the one we shall take. Evidently the particle is not exactly spherical; the axial ratio is probably between 1·1 and 1·2.

Providing that we accept the interpretation of the data as correct, there is no doubt that the X-ray method is the most accurate for estimating the size of bushy stunt virus, since the measurement of lattice edge can be made with an accuracy of 1%. It will have been noticed, however, that at a number of stages appeal has had to be made to plausibility, e.g. in assuming that the unit at each corner and cube centre in the lattice is a single virus particle and not a group. An independent method, even if less precise, is desirable, and this is provided by the sedimentation and diffusion experiments.

Lauffer & Stanley (1940) tabulate a large number of satisfactorily consistent determinations of sedimentation constant by themselves and other workers, which average to $131{\cdot}5 \pm 0{\cdot}8 \times 10^{-13}$. Neurath & Cooper (1940) have determined the diffusion constant to be $1{\cdot}15 \pm 0{\cdot}12 \times 10^{-7}$, and this value has been confirmed by Lauffer (1942) and Markham & Smith (unpublished). Knowing both diffusion and sedimentation constants, and also the partial specific volume of the dry virus (0·739, McFarlane & Kekwick, 1938), we are in a position to determine the degree of hydration in solution, and both the hydrated and unhydrated diameters, from the formulae of § VII. We deduce for the unhydrated particle diameter $29{\cdot}2 \pm 1{\cdot}2$ mμ and molecular weight $10{\cdot}65 \pm 1{\cdot}1 \times 10^6$, while for the hydrated particle diameter $= 37{\cdot}1 \pm 3{\cdot}8$ mμ and molecular weight $= 18{\cdot}8 \pm 5{\cdot}0 \times 10^6$. The degree of hydration is $w = 0{\cdot}76 \pm 0{\cdot}3$ g. water per gram dry virus. According to Bawden & Pirie's estimate that the wet crystals contain 55% of water, the degree of hydration in the crystal is $55/45 = 1{\cdot}22$ g. water per gram dry virus, i.e. the degree of hydration of the particles in solution is probably less, though not certainly less, than the hydration in the wet crystal.

McFarlane & Kekwick (1938) found a somewhat higher sedimentation constant, namely, 146×10^{-13}. A more serious discrepancy concerns the diffusion constant. McFarlane & Kekwick also made a sedimentation equilibrium experiment, which, since it determines s/D may be used to deduce D if s is known. Dr Kekwick kindly gave us the necessary numerical data from which we calculated $s/D = 7{\cdot}40 \pm 0{\cdot}14 \times 10^{-5}$, whence using $s_{20} = 146 \times 10^{-13}$, $D = 1{\cdot}97 \pm 0{\cdot}04 \times 10^{-7}$; or using $s_{20} = 131{\cdot}5 \times 10^{-13}$, $D = 1{\cdot}78 \pm 0{\cdot}04 \times 10^{-7}$. The standard deviations attached to these figures are derived from the mutual consistency of three runs of the sedimentation equilibrium centrifuge. Evidently there is a systematic error in either this experiment, or in Neurath & Cooper's experiment, or in both, since the results differ by an amount much in excess of the errors deduced for each method by the internal consistency of repetition experiments. McFarlane & Kekwick's data lead to an estimate for the molecular weight of the unhydrated virus of about 7×10^6 (7·6 calculated by the authors, $6{\cdot}88 \pm 0{\cdot}15$ recalculated by ourselves by a slightly different method), and indicate further no hydration of the molecule in solution. Since the molecular weight deduced from a combination of Lauffer & Stanley's sedimentation constant with Neurath & Cooper's diffusion constant agrees better with the X-ray diffraction results, we accept the value $s_{20} = 131{\cdot}5 \times 10^{-13}$ and $D_{20} = 1{\cdot}15 \times 10^{-7}$ in preference to McFarlane & Kekwick's values.

We deduce then that the unhydrated virus, with a partial specific volume 0·739, has a molecular weight $10{\cdot}65 \times 10^6$, an average diameter 29·2 mμ and an axial ratio probably not exceeding 1·2. In solution this hydrates to the extent of $0{\cdot}76 \pm 0{\cdot}3$ g. water per gram dry virus, and assumes a molecular weight (hydrated) of $18{\cdot}8 \pm 5{\cdot}0 \times 10^6$ and a mean diameter of $37{\cdot}1 \pm 3{\cdot}8$ mμ, the partial specific volume of the hydrated virus being $0{\cdot}853 \pm 0{\cdot}03$.

Tobacco mosaic virus

Tobacco mosaic virus particles are rod-shaped, as had been known from indirect evidence for a long time (Takahashi & Rawlins, 1933) and has recently been convincingly demonstrated by the electron microscope. The most accurate information about the size comes, as in the case of bushy stunt virus, from X-ray diffraction data. Bernal & Fankuchen (1941*a*) find that in a dry gel the particles lie with their axes 15·2 mμ apart, this presumably being the particle diameter. The length is a variable quantity, probably being different in different preparations of the virus. Kausche, Pfankuch & Ruska (1939) found the length to be usually a multiple of 300 mμ or possibly 150 mμ. Stanley & Anderson (1941), also using the electron microscope, found that 70% of the material in their least aggregated preparation was in particles of lengths 280 mμ ± 7%. It is quite likely that the molecule, in the sense of the smallest particle having the properties of the virus, may be considerably smaller than this; however, taking this length to be that of the molecule in the absence of other data we deduce the molecular weight of a cylindrical particle 15·2 mμ diameter and 280 mμ long and with partial specific volume 0·73 (Bawden & Pirie, 1937) to be $41{\cdot}94 \times 10^6$.

Prior to the determination of length by the electron microscope, measurements of viscosity had been used to determine the ratio of length to breadth, using formulae such as that of Kuhn,

$$\eta/\eta_0 - 1 = G\{2{\cdot}5 + \tfrac{1}{16}(L/d)^2\},$$

G being the volume of dissolved substance, including any water attached to it, per unit volume of solution and L/d the ratio of length to breadth. Rather than combining this with a sedimentation or diffusion measurement, as has usually been done in order to determine L and d separately, it seems preferable to use the X-ray diffraction data for the width. The dry diameter is 15·2 mμ. If hydration occurs to the extent of w g. of water of density ρ with 1 g. of dry virus of specific volume $V_0 = 0{\cdot}73$, then we obtain

$$\eta/\eta_0 - 1 = G_0\{2{\cdot}5(1 + w/V_0\rho) + \tfrac{1}{16}(L_0/d_0)^2\}$$

(cp. § IX). Since the first term is small compared with the second for a highly elongated particle, the degree of hydration has little result on the viscosity. Using Lauffer's (1938) value of $(\eta/\eta_0 - 1)/G_0 = 57{\cdot}9/0{\cdot}73 = 79{\cdot}4$, and $d_0 = 15{\cdot}2$ mμ, we deduce that the length L_0 of the particle is 532 mμ.

This estimate will be considerably increased if allowance is made for the alignment of the particles which probably takes place in the viscometer, since Kuhn's formula is deduced for randomly orientated rods.

The length deduced from viscosity measurements will be an average length, and therefore naturally exceeds the electron microscope figure of 280 mμ which was not an average length but the most probable length of the unaggregated virus.

We have now to see how far these lengths and widths are consistent with sedimentation and diffusion experiments. In sedimentation experiments separate boundaries are obtained for aggregated and unaggregated virus; the boundary sedimenting at 193×10^{-13}

(Lauffer, 1940) presumably corresponds to the length 280 mμ of the unaggregated virus. We have therefore calculated the anticipated sedimentation constant for several different degrees of hydration w, taking the unhydrated molecular weight to be $41{\cdot}94 \times 10^6$ and taking the length to be 280 mμ and the diameter $15{\cdot}2 \times \left(\frac{0{\cdot}73+w}{0{\cdot}73}\right)^{\frac{1}{3}}$ mμ. The sphere of equal volume has radius $r = 22{\cdot}98 \times \left(\frac{0{\cdot}73+w}{0{\cdot}73}\right)^{\frac{1}{3}}$ mμ while the axial ratio is $18{\cdot}44 \times \left(\frac{0{\cdot}73+w}{0{\cdot}73}\right)^{-\frac{1}{3}}$. The frictional coefficient is taken as $6\pi\eta Nrf/f_0$, where f/f_0 is the frictional ratio corresponding to the given axial ratio, and is calculated from Perrin's (1936) formula, tabulated in § IX.

By these means we deduce the following table relating anticipated sedimentation constant with degree of hydration. It is evident that the experimental value of $s_{20} = 193 \times 10^{-13}$ suggests w between 1 and 1·5 g. of water per gram of dry virus.

In similar fashion we have calculated the diffusion constant at 25° C. for comparison with Neurath & Saum's determination (1938). In this determination no separation of unaggregated and aggregated virus can be made, so that the figure given is an average figure. We have therefore used the length 532 mμ suggested by the viscosity experiments in preference to the length 280 mμ of the unaggregated virus; otherwise the calculation follows the same lines and gives the following table (Table 4) relating anticipated diffusion

Table 4. *The degree of hydration of tobacco mosaic virus*

	Unhydrated size taken as $280 \times 15{\cdot}2$ mμ				
Hydration w (g. water per g.)	0	0·5	1·0	1·5	2·0
Width in solution (mμ)	15·2	19·7	23·4	26·6	29·4
Axial ratio	18·4	14·2	12·0	10·6	9·5
f/f_0	1·94	1·75	1·64	1·57	1·52
Sedimentation constant $s_{20}(\times 10^{-13})$	222·2	207·2	196·5	187·0	182·6
	Unhydrated size taken as $532 \times 15{\cdot}2$ mμ				
Hydration w (g. water per g.)	0	0·5	1·0	1·5	2·0
Axial ratio	35·0	27·0	22·7	20·0	18·1
f/f_0	2·52	2·25	2·11	2·00	1·92
Diffusion constant $D_{25}(\times 10^{-8})$	3·40	3·20	3·06	2·96	2·88

constant with degree of hydration. Agreement with Neurath & Saum's determination $D_{25} = 3 \times 10^{-8}$ is obtained at w between 1 and 1·5.

Thus the sedimentation and diffusion experiments can both be accounted for on the assumption of a degree of hydration of 1–1·5 g. of water per gram of dry virus. This would increase the diameter of the particles in solution to about 25 mμ.

Undue weight should not be attached to this calculation of the degree of hydration, since it depends on the applicability of Perrin's formula for randomly orientated ellipsoids to the present case. It may however be taken to suggest that tobacco mosaic virus, like the other viruses here considered, is hydrated in solution.

XI. Summary and conclusions

In this review we have given an account of the various methods which are available to determine the size of virus particles. In § IV we have endeavoured to bring the ultrafiltration method into agreement with other methods by suggesting a different factor for converting pore size to virus size from the factors commonly used. Throughout we have recognized the probability that most viruses are hydrated in solution and have dis-

tinguished between the size and molecular weight in solution and the size and molecular weight when dried.

In § VII we have given formulae suitable for interpreting centrifugation and diffusion data when the possibility of hydration is contemplated.

It is evident that this complication, added to that of shape, makes it necessary for several measurements by different methods to be made before one can claim to know the size of a virus. For this reason, only in the cases of three viruses have we thought the data sufficiently adequate to enable the size and shape and molecular weight of the virus, both dry and hydrated, to be stated. These three viruses, tobacco mosaic, tomato bushy stunt and vaccinia respectively, are separately discussed in § X.

It will be clear from the preceding sections that, while the position regarding our knowledge of the absolute sizes of viruses is far from satisfactory, there has been amassed a large amount of data bearing on this subject. We should, however, point out that we have found it necessary to select what we consider to be the best experimental data in some cases and that there may be conflicting ideas expressed by various authorities. Frampton (1942) has studied the electron microscope photographs published by Stanley & Anderson (1941) and Anderson & Stanley (1941) and arrives at an entirely different estimate of the length of tobacco mosaic virus. Kausche, Pfankuch & Ruska (1939) reported one value for the length of this virus which is approximately half that given by Stanley & Anderson. Electron photomicrographs published by von Ardenne (1940) and Holmes (1941) for what are probably strains of the same virus, also suggest that the values given should not be taken as absolute. Frampton (1939 *a*, *b*), on the basis of diffusion and viscosity experiments and the stream birefringence of this virus, has suggested previously that it forms a gel at any concentration and therefore cannot be said to have a size. Lauffer (1940) has given reasons for supposing this argument to be incorrect. Bernal & Fankuchen (1941 *a*) have discussed the possibility of tobacco mosaic virus particles being shorter than the value taken from Kausche *et al.* (1939) and conclude that in the plant itself the particle may be as short as 100 mμ.

In obtaining values of size and shape from electron microscope data we have made the assumptions, which may not be correct, that long, thin viruses shrink in width rather than in length on drying and that almost spherical viruses contract approximately evenly in all directions. At the moment there would seem to be no method of proving or disproving the truth of these assumptions, but we believe it unlikely that drying will result in such a gross change in shape that it would invalidate our calculations. For instance, in the case of haemocyanin from *Helix pomatia*, it seems improbable that, on drying, an already anhydrous ellipsoidal molecule of $66 \times 15{\cdot}3^2$ mμ would contract in length and expand in width to form a sphere of some 24 mμ diameter.

In our treatment of hydration we have found it necessary to regard the density and volume of 'bound' water as being the same as that of water in bulk, which may not be entirely true. However, we regard the total volume occupied by water in cases of great hydration, as shown by tomato bushy stunt virus, as being not markedly smaller than that of the same mass of free water. It is, nevertheless, a well-established fact that in certain cases, gelatin for example (Svedberg, 1924), a small contraction in volume does take place when dry protein is added to water. This phenomenon does not, however, necessitate the assumption that the water of hydration is denser than ordinary water, and can be explained in other ways.

The viscosity of solutions of viruses, especially the rod-shaped plant viruses, has attracted much attention as a method of finding frictional and axial ratios of viruses (Frampton, 1939 *a*, *b*; Lauffer, 1938; Loring, 1938; Neurath, Cooper, Sharp, Taylor, Beard & Beard, 1941; Robinson, 1939 *a*, *b*; Stanley, 1939), but, in addition to the lack of experimental verification of the formulae used, in many cases (Robinson, 1939 *a*, *b*; Frampton, 1939 *a*, *b*) the formulae have been applied to experimental results obtained in circumstances which exclude the fundamental postulates on which the formulae are based. For this reason we have omitted a detailed discussion of such methods.

It would appear that in order to obtain evidence as to the size of a virus it is desirable to study the virus in as purified a form as possible and also to show that when 'homogeneous' preparations are obtained, they do not consist merely of macromolecular substances contaminated with a small quantity of virus. Furthermore it is desirable to obtain at least sufficient data to enable one to assess both size and shape of the particles rather than to assume some shape or some density value which may be incorrect.

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